SYNTHESIS, CHARACTERISATION AND EVALUATION OF SLOW NITROGEN RELEASE ORGANIC SOIL CONDITIONERS FROM SOUTH AFRICAN TECHNICAL LIGNINS

by

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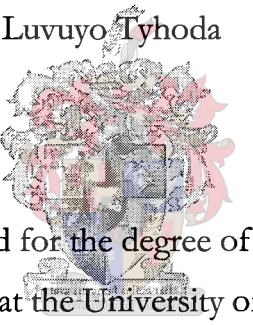

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Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and I have not previously in its entirety or in part submitted it to any university for a degree.

Signature:.....

Date:..... March 2008.....

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Abstract

Today there is renewed interest in using technical lignins and similar ligneous materials as soil fertilising materials as they are available in large quantities from the pulping and agricultural industries. In addition, excessive application of inorganic fertilisers results in ground water pollution, especially in sandy soils through leaching of nitrogen. Lignin is an important precursor of humic material in soils and with modification, can be used as a raw material to produce slow nitrogen release fertilisers (N-lignins). This can be done using a reaction process called oxidative ammonolysis. This process has been developed over the years by the Institute of Plant and Wood Chemistry of the Technical University of Dresden in Germany, and a patent has been obtained for an ambient pressure based, oxidative ammonolysis. The patented product obtained from this process which is prepared from low-grade brown coal (due to its similarity to lignin) is trade named NOVIHUM™. It is a slow nitrogen release and long lasting organo-mineral fertiliser which has similar properties as natural humic substances.

This project focussed on the characterisation and use of industrial residues such as technical lignins and similar ligneous materials which are produced by the South African pulping and agricultural industries, and on the production of N-lignins according to the patented NOVIHUM™ technology. Three technical lignins, one derived from the sulphite pulping process (a calcium lignosulphonate), the second from sugar cane bagasse (Sucrolin), and a third a, partially stripped sugar cane bagasse (a lignocellulosic residue with a low lignin content) were subjected to oxidative ammonolysis. A Kraft lignin, Indulin AT, from Westvaco, USA was used for comparison. The N-lignin products obtained were characterised and evaluated in plant growth trials.

Wet chemical methods and a combination of instrumental techniques such as elemental analysis (CNS), Curie-point Pyrolysis GCMS, HPLC and ^{31}P NMR spectroscopy were

used to characterise the raw materials prior to oxidative ammonolysis, as well as the N-lignin products obtained afterwards. To gain an understanding of the nature in which the nitrogen is bonded to the lignin macromolecules, a combination of analytical techniques i.e. Curie-Point Pyrolysis GCMS, X-ray photoelectron spectroscopy (XPS) as well as ^{15}N CPMAS NMR spectroscopy were used to study the N-containing lignin substructures.

The highest nitrogen content was obtained with the Sucrolin and the lowest with the bagasse lignocellulosic residue. All the N-lignin products, except the N-bagasse lignocellulosic residue were within range (3-5% N) obtained with N-lignin reported in literature, confirming the feasibility of using some of these materials as raw materials for N-lignins. Elemental analysis of the products revealed nitrogen contents of the N-lignin products in the following order: N-Sucrolin (6.25% N; 9.8 C/N ratio), N-calcium lignosulphonate (3.36% N; 12.6 C/N ratio), N-modified bagasse lignocellulosic residue (2.64% N; 19.50 C/N ratio) and N-Indulin AT (3.07% N; 19.50 C/N ratio). The nitrogen content of N-Sucrolin was higher than that of NOVIHUM™ (5.5% N; 11.51 C/N ratio) the reference material against which all the products were evaluated. The total nitrogen of the bagasse lignocellulosic residue could be increased to 3.59%; 12.90 C/N ratio after pre-treatment with hydrogen peroxide and oxidative ammonolysis in oxygen instead of air. In the case of Sucrolin, such a pre-activation was not required as the N content was already high. Too high N-contents result in too narrow C/N ratios which then necessitate addition of N-lignin only in smaller quantities to the soil, which in turn reduces the soil improving benefits that could be derived not only from the nitrogen, but also from the humus properties of NOVIHUM™-type materials.

In nursery pot tests on yellow mustard, *Sinapis alba,* grown on a nutrient deficient soil treated with the N-lignins over two growth periods, the N-Sucrolin resulted in a cumulative dry mass yield (4.46 t/ha) that was similar to NOVIHUM™ (4.68 t/ha), the reference product. The N-bagasse lignocellulosic residue resulted in a lower cumulative dry mass yield (3.75 t/ha), as a result of a lower N content due to its considerable cellulose content. The N-calcium lignosulphonate yielded the lowest dry matter (0.91 t/ha) which was only 19.4% of that of NOVIHUM™ as a result of stunted growth. It

also had high solubility due to its high sulphur content causing it to rise and precipitate on the soil surface, making it susceptible to surface erosion. The cause of the stunted growth, although not evaluated in this study could be linked to phytotoxic effects due to its high reducing sugar content (mainly xylose). In addition, phytohormonal analysis of the leaves of yellow mustard, *Sinapis alba,* indicated that the low yield was not associated with the N-content. In tree seedling (*Eucalyptus marcarthurii* and *Acacia mearnsii*) experiments on acidic and saline soils, the N-Sucrolin gave yields similar to NOVIHUM™ in the acidic soil. In alkaline soil neither the NOVIHUM™ nor the N-Sucrolin could mitigate the effects of high pH. Further plant tests on rye grass, *Lollium perenae,* showed that a range of 0.3% to 1% (mass % N-lignin/ mass % soil) led to better nitrogen efficiency compared to higher values. In addition, they showed that N-lignins, compared to easily soluble inorganic fertilisers, can reduce nitrogen leaching. Future studies on the use of calcium lignosulphonate should focus on phytotoxic compounds generated during oxidative ammonolysis.

Pyrolysis GCMS indicated that, when compared to the high-pressure technology, ammoxidation at ambient pressure and moderate temperature (80°C) resulted only in moderate modifications of the lignin polymer network. Oxidation lead to formation of COOH groups resulting in the formation of ammonia salts and highly reactive quinoide structures. The latter could have further reacted with NH_3 to give a variety of N-containing substructures or even polymeric N-containing compounds. Hydrogen peroxide pre-activation, indicated that these relatively strong oxidative conditions resulted in the loss of some portions of the "weakly bound organic matter". New insights into the nature of N-binding forms of N containing compounds in N-lignins were provided through a combination of analytical techniques i.e. Curie-Point Pyrolysis GCMS, X-ray Photoelectron Spectroscopy (XPS) and ^{15}N CPMAS NMR.

Opsomming

Daar is vandag hernude belangstelling in tegniese ligniene en soortgelyke lignienagtige materiale om hulle as grondbemestingstowwe te gebruik, hoofsaaklik omdat hulle in groot hoeveelhede in die pulp- en landbouindustrië beskikbaar is. Daarmee saam lei die oormatige aanwending van anorganiese misstowwe tot grondwaterbesoedeling, veral in sandgronde as gevolg van uitloging van stikstof. Lignien is 'n belangrike voorloper van humusagtige materiaal in grond en kan mbv chemiese modifikasie as grondstof vir stadigvrystellende stikstofmisstowwe (N-ligniene) gebruik word. Dit kan met 'n proses, genoem oksidatiewe ammonolise, uitgevoer word. Hierdie proses is die afgelope tyd deur die Plant en Houtchemie-instituut aan die Tegniese Universiteit van Dresden in Duitsland ontwikkel en die oksidatiewe ammonolise by atmosferiese druk is gepatenteer. Die gepatenteerde produk wat mbv hierdie proses vanaf lae-graad bruinkool (agv sy soortgelyke eienskappe as lignien), vervaardig is, word in die handel NOVIHUM™ genoem. As 'n langwerkende, organominerale misstof besit dit soortgelyke eienskappe as natuurlike humusagtige stowwe.

Hierdie ondersoek fokus op die karakterisering en gebruik van industriële residue soos tegniese ligniene en soortgelyke lignienagtige materiale wat deur die Suid Afrikaanse pulp- en landbouindustrië produseer word, en om N-ligniene volgens die gepatenteerde NOVIHUM™ tegnologie daarvan te vervaardig. Drie tegniese ligniene, een afkomstig van die sulfietverpulpingsproses ('n kalsiumlignosulfonaat), die tweede van suikerrietbagasse (Sucrolin) en 'n derde, 'n gedeeltelik verwyderde suikerrietbagasse dws 'n lignosellulose-agtige residu met 'n lae lignieninhoud, is aan oksidatiewe ammonolise onderwerp. Indulin AT, 'n Kraftlignien, afkomstig van die firma Westvaco, VSA, is as verwysing gebruik. Die N-lignienprodukte wat verkry is, is gekarakteriseer en dmv plantgroeitoetse evalueer.

Nat chemiese metodes en 'n kombinasie van tegnieke soos elementanalise (CNS), Curie-punt Pirolise GCMS, HPLC en ^{31}P KMR spektroskopie is aangewend om die rumateriale vóór oksidatiewe ammonolise en die gesintetiseerde N-ligniene te karakteriseer. 'n Kombinasie van analitiese tegnieke soos Curie-Punt Pirolise GCMS, X-straal foto-elektronspektroskopie (XPS) sowel as ^{15}N CPMAS KMR spektroskopie is gebruik om vas te stel op watter wyses stikstof aan die lignienmakromolekules gebind is.

Die hoogste stikstofinhoud is met Sucrolin en die laagste met die bagasse lignosellulose-residu verkry. Alle N-lignienprodukte behalwe die N-bagasse lignosellulose-residu, het 'n stikstofinhoud van 3-5% gehad, soos wat in die literatuur vir N-ligniene gerapporteer is en wat die geskiktheid van hierdie rumateriale bevestig het. Die elementanalise van die N-ligniene het die volgende stikstofinhoud gelewer: N-Sucrolin (6.25% N; 9.8 C/N verhouding), N-kalsiumlignosulfonaat (3.36% N; 12.6 C/N verhouding), N-gemodifiseerde, bagasselignosellulose-residu (2.64% N; 19.50 C/N verhouding) en N-Indulin AT (3.07% N; 19.50 C/N verhouding). Die stikstofinhoud van N-Sucrolin was hoër as van NOVIHUM™ (5.5% N; 11.51 C/N verhouding), die materiaal waarmee alle produkte vergelyk is. Na voorbehandeling met waterstofperoksied en oksidatiewe ammonolise met suurstof ipv van lug, kon die totale stikstofinhoud van die bagasselignosellulose-residu na 3.59%; 12.90 C/N verhouding verhoog word. Hierdie voorbehandeling was nie vir Sucrolin nodig nie aangesien die stikstofinhoud alreeds hoog was. Te hoë stikstofinhoud lei tot te lae C/N verhoudings, wat kleiner toevoegings N-lignien aan grond vereis en wat op sy beurt nie net die grondverbeteringsvoordele van stikstof maar ook die humuseienskappe van NOVIHUM™-tipe materiale, verminder.

In kwekery-pottoetse wat met geel mosterd, *Sinapis alba*, op nutriënt-arme grond wat met N-ligniene behandel is en oor twee groeiperiodes uitgevoer is, is met N-Sucrolin 'n kumulatiewe droë massa-opbrengs van 4.46 t/ha verkry, soortgelyk aan 4.68 t/ha met NOVIHUM™, die verwysingsproduk.

Die N-bagasse lignosellulose residu het 'n laer kumulatiewe droë massaopbrengs (3.75 t/ha) opgelewer agv die laer N-inhoud soos veroorsaak deur die beduidende sellulose-inhoud daarin. Agv van vertraagde plantgroei het N-kalsiumlignosulfonaat die laagste opbrengs (0.91 t/ha), ongeveer 19.4% van wat met NOVIHUM™ verkry is, gelewer. Agv sy hoë oplosbaarheid (vanweë sy hoë swawelinhoud), het dié N-lignien na die grondoppervlak beweeg en daar gepresipiteer, wat dit dan aan gronderosie sou kon blootgestel het. Alhoewel vertraagde groei nie ondersoek is nie, kan dit moontlik aan fitotoksiese effekte wat met hoë reduserende suikerinhoud (veral xilose) geassosieer word, toegeskryf word. Dmv fitohormoon-analise van die blare van geel mosterd, *Sinapis alba,* is vasgestel dat lae opbrengs nie met N-inhoud geassosieer word nie. In eksperimente met boomsaailinge (*Eucalyptus marcarthurii* en *Acacia mearnsii*) met suur en alkaliese gronde het N-Sucrolin soortgelyke opbrengste as met NOVIHUM™ in suurgronde gegee. In alkaliese en suurgronde kon beide NOVIHUM™ en N-Sucrolin nie die effekte van hoë pH verlaag nie. Verdere planttoetse met roggras, *Lollium perenae*, het getoon dat hoeveelhede van 0.3% tot 1% (massa % N-lignien/ massa % grond) tot beter stikstofeffektiwiteit kon lei as met hoër konsentrasies. Die toetse het ook aangetoon dat, soos by maklik oplosbare anorganiese misstowwe, uitloging by N-ligniene verminder kan word. Verdere studie op die gebruik van kalsiumlignosulfonaat behoort op die fitotoksiese verbindings wat tydens oksidatiewe ammonolise ontstaan te fokus.

Met pirolise GCMS kon vasgestel word dat ammoksidasie by atmosferiese druk en matige temperatuur (80°C) slegs tot klein veranderings in die lignienstruktuur gelei as wat met die hoëdruktegnologie die geval was. Oksidasie het tot die vorming van COOH-groepe gelei wat tot die vorming van ammoniumsoute en baie reaktiewe kinoïedestrukture aanleiding gegee het. Laasgenoemde kon verder met NH_3 reageer het om 'n verskeidenheid N-bevattende substrukture of selfs polimeriese, N-bevattende verbindings te lewer. Voorafbehandeling met waterstofperoksied het aangetoon dat hierdie relatief sterk, oksiderende toestande tot die verlies van "swakgebinde organiese materiaal" gelei het. Nuwe insig oor die aard van die N-bindingsvorms van N-bevattende verbindings in N-ligniene is dmv 'n kombinasie van analitiese tegnieke soos

Curie-Punt Pirolise GCMS, X-straal Foto-elektronspektroskopie (XPS) en ^{15}N CPMAS KMR spektroskopie verkry.

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List of acronyms

ABA	Abscisic acid
AOX	Sum of halogenated organic compounds'
BOD	Biological oxygen demand
CEC	Cation exchange capacity
CPMAS NMR	Cross Polarization Magic Angle Spinning Nuclear Magnetic Resonance
CRFs	controlled release fertilisers
FA	Fulvic acid
FAO	Food and Agriculture Organization
GEFSOC	Global Environment Facility Soil Organic Carbon
GC/MS	Gas chromatography/Mass Spectroscopy
GLASOD	Global Assessment of Soil Degradation
HA	Humic acid
HIV/Aids	Acquired immune deficiency syndrome/ human immunodeficiency virus
HPLC	High pressure liquid chromatography
ISRIC	International Soil Reference and Information Centre
IUCN	International Union for the Conservation of Nature and Natural Resources
NBI	National Botanical Institute
NMR	Nuclear magetic resonance
SOC	Soil organic carbon
SOM	Soil organic matter
SRFs	Slow release fertilisers
UN	United Nations
UNCCD	United Nations Convention to Combat Desertification
UNEP	United Nations Environment Programme

WRC	Water Research Commission
WMO	World Meteorological Organization
XPS	X-ray photoelectron spectroscopy

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1.0 Background

Lignin based, high grade humic materials are produced through a reaction process called oxidative ammonolysis (OA), ammoxidation, ammonoxidation or N-modification. The process is characterised by the reaction of an oxygen rich source such as air or pure oxygen, with a lignin based polymer or a similar carbonaceous material. Oxidative ammonolysis takes place in an alkaline medium in the presence of ammonia, under defined laboratory conditions such as heat, time and pressure.

The products obtained are called *N*-modified lignins or *N*-lignins. However, as other lignin based carbonaceous materials such as low grade coal (brown coal or lignite) can also be used; a distinction is made when reference is made to these particular raw materials. The technical procedure developed for the production of *N*-lignins or *N*-lignites enables the specific synthesis of products in which the nitrogen is differently bonded to the lignin or lignin based polymer and hence released to plants over extended periods of time. The following classification is used to designate different forms of nitrogen bonded to the lignin macromolecule as found in *N*-lignins:

- ammonium nitrogen (available to plants immediately),
- amide nitrogen (available to plants over the mid-term), and
- strongly bonded nitrogen (available to plants over a longer period).

The stepwise mineralisation of the nitrogen from *N*-lignins is a unique and a singly, most important feature of these materials as it results in the reduction of nitrogen leaching into the environment in the form of nitrates. This is often the case with easily soluble inorganic fertilisers especially when (1) applied on sandy soils which have very limited sorption capacities, (2) sites under intensive irrigation or (3) over application of fertilisers. The nitrogen content, carbon nitrogen ratio (C:N ratio), the percentages of the different types of chemically bonded nitrogen groups, cation exchange capacity

(CEC) as well as a distinct content of humic acid substructures are the main criteria for the evaluation of the *N*-lignins. Especially the proper distribution of the nitrogen binding forms is of great importance to achieve a well-balanced, long-term fertilisation of certain soils since differently bonded nitrogen becomes plant-available at different rates.

At the Institute of Plant and Wood Chemistry of the Technical University of Dresden, Germany, the process of oxidative ammonolysis has been extensively studied since the 1970s. Intensive research on different process conditions and materials from different sources has been undertaken, leading to a patent of the technology being obtained for the process (Fischer et al. 1993), as well as the novel *N*-modified products developed from it, trade named NOVIHUM™ (Fischer et al. 2000). A pilot plant was commissioned in 2000 to produce sufficient quantities for large scale evaluation of the material in field trial applications. The pilot plant also served as a base for the development of the product as well as the manufacturing process for industrial production of NOVIHUM™. A company trading under the same name has since been formed in 2000 in Freienhufen, Grossraeschen, Germany.

In general, NOVIHUM™ is a humus fertiliser which is rich in nitrogen and low in noxious materials and is produced from natural materials e.g. low grade coal and technical lignins by oxidative ammonolysis. It improves the soil on a long-term basis, promotes growth of plants and improves the quality of harvested products. The main and possible areas of applications are listed below:

- Cultivation of soils in deserts and semi-deserts
- Afforestation of Karst areas
- Re-cultivation of land after surface mining activities
- Rehabilitation of problematic areas (industrial dumps, areas with abandoned waste etc)
- Production of vegetables with low NO_3^- level (bio-vegetables)

- Cultivation of plants for forestry
- Application in drinking water protection areas
- Cultivation of ornamental plants, grassy roofing, production of pre-fabricated roll-grass for sports fields and parks.

The key feature of the process on *N*-modification of lignin based materials is the total recycling of agricultural wastes and industrial residues into a unique and valuable fertiliser that can be safely used in a range of agricultural, soil rehabilitation and horticultural applications. This is a sustainable method of recycling industrial residues into a useful and valuable non-food, non-fuel product. The product is flexible as a range of nutrients and additives can be included in the product as required for different applications.

1.2 Study aims

Screening studies aimed at establishing the potential of lignin based raw materials available in South Africa for the production of high-grade humic materials, including the use of low grade coal occurring in South Africa, had earlier been carried out (Tyhoda 2003). The materials were evaluated in terms of their reactivity with ammonia and hence the total nitrogen they could incorporate. However the modification was carried out using an earlier method for modifying lignin based materials which employs oxygen under high–pressure. Nevertheless, the results obtained from these studies gave insight into which materials could be used to produce high grade humic materials using the patented technology by which NOVIHUM™ is produced. In addition, the process was chosen because of its simplicity and low material requirements.

The objective of the current work was to modify the selected South African industrial wastes and residues to produce N-lignins using the said technology and evaluate the potential use of the products for soil improvements. It is further aimed that the performance of the products obtained are evaluated in field trial applications, to establish the claimed potential of the technology to produce materials which can

improve soil properties of nutrient deficient soils as well as its ability to reduce nitrate leaching to ground water.

1.2.1 Scientific approach

1.2.1.1 Characterisation of the raw materials

The selected feed stocks were characterised for physical and chemical properties in order to determine the most suitable route for the manufacture of N-lignins. Characterisation included the determination of the following:

- Moisture contents
- Ash content
- Carbon, nitrogen and sulphur (CNS) composition
- Functional group contents i.e.:
  - (i) Methoxyl
  - (ii) Carboxyl
  - (iii) Carbonyl and
  - (iv) Hydroxyl group content
- Presence of monosaccharides (with particular interest in xylose) by wet chemical methods and HPLC.

A combination of analytical techniques such as NMR and Curie-point Pyrolysis GCMS was used to elucidate the structure and functional groups of the various raw materials.

1.2.1.2 Production of the N-lignins

Oxidative ammonolysis of the selected raw material feed stocks was carried out on a small scale using a laboratory scale reactor to provide samples to identify the most effective and suitable product for growth.

1.2.1.3 Laboratory evaluation of the N-lignins

The following criteria were assessed:

- total incorporated nitrogen in the product

- C:N ratios
- the proportions of the various forms of bonded nitrogen
- the structure of nitrogen containing lignin compounds using a combination of analytical techniques i.e. NMR, XPS and Pyrolysis GCMS.

Changes in functional group contents of the materials before and after modification were compared.

1.2.1.4 Performance evaluation of the N-lignins on crops

Biomass production of mustard seed *Sinapis alba* in soil treated with nitrogen modified technical lignins was evaluated in a pot experiment in a green house over two growth periods and compared with urea and NOVIHUM™. Growth parameters such as height, dry matter yield and nitrogen content were investigated.

1.2.1.5 Phytohormonal activity on crops

The physiological mechanism underlying performance of the different materials as related to yield was investigated. This was done by studying plant hormones abscisic acid and isopentenyladenosine in the leaves of mustard seed, *Sinapis alba,* grown on soil fertilised with N-lignin products. Urea and NOVIHUM™ were used for comparison.

1.2.1.6 Slow nitrogen release evaluations of N-lignins

NOVIHUM™ was tested in a green house experiment on rye grass against ammonium sulphate to establish its release behaviour and its effect on nitrogen use efficiency on crops. The slow nitrogen release capability was evaluated through leaching experiments, by measuring the amount of nitrogen in percolation water, as well as nutrient recovery by the plants under various application rates and soil types.

1.2.1.7 Large scale production and testing of N-lignins on tree seedlings

The most promising slow nitrogen release fertiliser product from the biomass production evaluatory tests (section 1.2.1.4) was produced in quantities of 20-25 kg for

larger scale testing. Its performance in growing tree seedlings in a soil rehabilitation effort of two distinctly different nutrient deficient soils i.e. an acidic soil resembling the situation found in post-mining landscapes and an alkaline soil was evaluated against NOVIHUM™ and conventional inorganic fertiliser ammonium sulphate in a nursery environment. *Eucalyptus marcarthurii*, a non-nitrogen fixer and *Acacia mearnsii* a nitrogen fixer were used as test species.

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2.0 Literature review

2.0 The problem of land degradation

Past and present human intervention in the utilization and manipulation of environmental resources are having unanticipated consequences. The often-indiscriminate destruction of forests and woodlands, and the spectre of land degradation resulting in decreased productivity with dire social consequences are generally recognized. The earth's soils are being washed away, rendered sterile or contaminated with toxic chemicals at a rate that cannot be sustained (Oldeman et al. 1991). "There is a growing realisation in national and multilateral institutions that not only many forms of economic development erode the environmental resources upon which they are based, but at the same time environmental degradation can undermine economic development" (Brundtland and Khalid 1987).

Land degradation is a complex issue as it encompasses physical, biological and socio-economic parameters. As such there are numerous terms and definitions that are used to define it, which sometimes are a source of confusion, misunderstanding, and misinterpretation. Most of the terms used in the literature are often disciplinary-oriented, and the definition of land degradation sometimes misinterpreted among disciplines. Some common terms used are soil degradation, land degradation, and desertification. While there is a clear distinction between 'soil' and 'land', and as such this discussion pays special attention to each of the terms, (the term land refers to an ecosystem comprising land, landscape, terrain, vegetation, water, climate), there is no clear distinction between the terms 'land degradation' and 'desertification'.

Barrow (1991) describes land degradation as the reduction in rank or status, for example, a degradation and/or loss of soil, or change to a simpler floral/faunal composition or a substitution of one organic form for a lower organic rank. The International Convention to Combat Desertification (UN 1994) describes "land

degradation" as the reduction or loss, in arid, semi-arid and dry sub-humid areas of the biological or economic productivity and complexity of rain fed cropland, irrigated cropland, or range, pasture, forest and woodlands resulting from land uses or from a process or combination of processes, including processes arising from human activities and habitation patterns, such as:

i. soil erosion caused by wind and/or water;
ii. deterioration of the physical, chemical and biological or
iii. economic properties of soil; and
iv. long-term loss of natural vegetation.

According to this definition, land degradation involves the processes *and* end results of both vegetation and soil degradation due to natural and/or anthropological factors. In as much as air and water are important components of the ecosystem whose quality also continues to decrease worldwide as a result of anthropogenic factors, a separate discussion will be given on them on a separate section dealing with fertilisers and the environment.

In general, land degradation and environmental degradation are primarily caused by drought, desertification, soil salinity and water logging, conversion of rangeland into cropland, uncontrolled expansion of rural settlements at the cost of arable land, pollution, military activities, unsustainable human activities of which the most common cited forms are over exploitation of resources such as over cultivation, overgrazing, deforestation, and poor irrigation practices. Land degradation manifests itself as the decline of the quality of air, land and water resources. In short, key aspects of land degradation are anthropogenic activities, soil degradation, drought and desertification. Each of these key aspects is discussed in subsequent sections below.

2.1 The key aspects of land degradation

2.1.1 Soil degradation

Soil degradation may be defined as: a reduction of the current and/ or future capability of the soil to produce, in terms of quantity, quality goods or services (Barrow 1991).

Statistics show that the total land area prone to soil degradation is estimated to be 2 billion ha, of which 562 Mha (29.7%) is agricultural land, 685 million ha (34.8%) is permanent pastures and 719 Mha (35.5%) is forest and woodland around the globe. The following map published by UNEP (Rekacewicz 1997) shows the general state of soil degradation on a global scale, which perhaps is in agreement with the figures mentioned above.

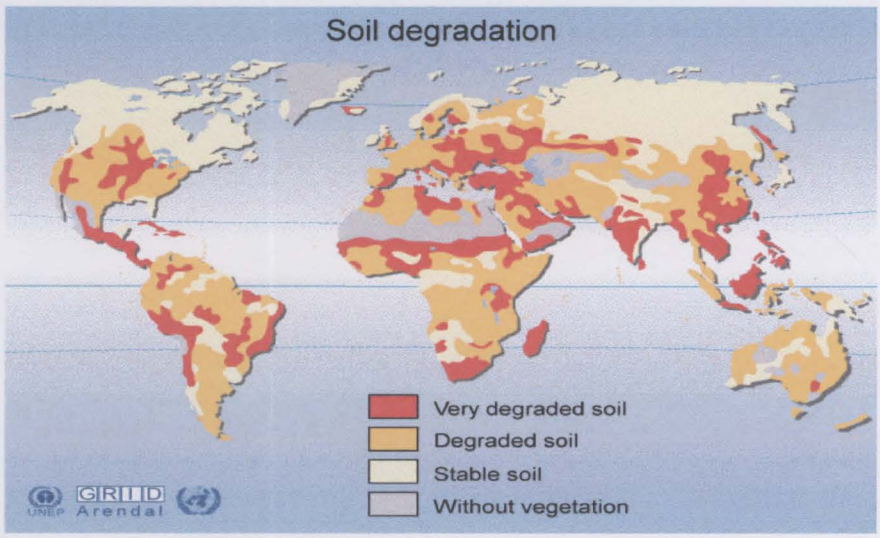

Figure 2-1: The global state of soil degradation

It clearly shows that much of the Earth is degraded, is being degraded or at risk of degradation; an observation also made by Barrow (1991). This is a clear indication that soil degradation is a serious issue of the modern era and will remain so during the 21st century as Lal (2001) also observed. Important among anthropogenic factors responsible for land degradation are land use, soil management, farming/cropping systems, land tenure, marketing, and institutional support. Soil degradation is accentuated by poverty, and can cause more serious problems on soils managed for subsistence agriculture with no external input than those managed under commercial agriculture with science based input. The main causes of soil degradation are classified below.

2.1.1.1 Soil erosion

In general, soil erosion is a natural process that entails weathering of the landscape and is largely controlled by climate, topography, soil vegetation cover and other eco-regional characteristics. The slow geologic erosion is a constructive process which has created vast tracts of fertile soils of alluvial flood plains and loess plateaus around the world. These soils with built-in soil fertility renewal mechanisms, have supported ancient civilizations (e.g., in the valleys of the Nile, Euphrates, Indus, Yangtze) and thriving cultures for millennia. In contrast, the accelerated soil erosion, exacerbated by anthropogenic perturbations, is a destructive process. It depletes soil fertility, degrades soil structure, reduces the effective rooting depth and destroys the most basic of all natural resources (Lal 2003). It has adverse economic and environmental impacts. Economic effects are due to loss of farm income due to on site and off site reduction of income and adverse impacts on crop/animal production. The environmental impacts are due to pollution of natural waters.

Each year, about 75 billion tons of soil is eroded from the world's terrestrial ecosystems, making soil erosion the greatest threat to providing food for a rapidly growing human population. The FAO estimates that 5-7 million ha of productive land are lost through erosion each year. Most agricultural land in the world is losing soil at rates ranging from 13 tons/ha/year to 40 tons/ha/year. Because soil is formed very slowly, this means that soil is being lost 13–40 times faster than the rate of renewal and sustainability (Pimentel and Kounang 1998). In Africa, it is estimated that about 320 million ha, or about one quarter of its dry lands are affected by different types of soil erosion (UNEP 1997). Other global hotspots as described by Reich and Eswaran (2004) include:

i. *China*: where 1.6 billion tons of soil per year washes into the Yellow River from China's Loess Plateau which has the highest rates of water erosion in the world.
ii. *United States of America*: where decades of water erosion degraded soil across Plains, although no-till stemmed losses.

iii. *Amazon:* where slash-and-burn agriculture in the Amazon exposes poor tropical soils that can sustain crops for only a few years before nutrients wash away.

iv. *Himalaya:* where overgrazing and deforestation have spurred widespread soil erosion in the lower Himalaya Mountains, where natural rates are already high because of monsoonal rains.

From a South African perspective it has been quoted in many publications that the country loses approximately 300 to 400 million tons of soil to soil erosion on an annual basis (Collins 2001). In addition, it is also estimated that South Africa loses 30 000 tonnes of nitrogen, 26000 tonnes of phosphorus and 36 000 tonnes of potassium on an annual basis (Hoffman and Todd 2000). The annual cost for replacing these nutrients is estimated to exceed R1.5 billion. A separate discussion on the extent and main causes of soil degradation South Africa follows in subsequent sections. The two main types of soil erosion are discussed the following subsections.

Erosion by water

Water erosion is by far the most important type of soil degradation occupying around 1094 million ha or 56% of the total area affected by human-induced soil degradation. It occurs in almost every country under a great variety of climatic and physical conditions and land use. It is usually seen as sheet erosion (a more or less uniform removal of a thin layer of topsoil), rill erosion (small channels in the field) or gully erosion (large channels, similar to incised rivers). As the topsoil is normally rich in nutrients, a relatively large amount of nutrients is lost together with the topsoil. This process may lead to an impoverishment of the soil. On very steep slopes, natural loss of topsoil may occur frequently.

Erosion by wind

Wind erosion is a soil degradation type which is defined as the uniform displacement of topsoil by wind action. It is a widespread phenomenon in arid and semi-arid climates but it also occurs under more humid conditions. In general, coarse-textured soils are more susceptible to wind erosion than fine-textured soils. Wind erosion is nearly

always caused by a decrease of the vegetation cover of the soil, either due to overgrazing or to removal of vegetation for domestic use or for agricultural purposes. In (semi-)arid climates natural wind erosion is often difficult to distinguish from human-induced wind erosion, but natural wind erosion is often aggravated by human activities. On a world scale the area affected by wind erosion occupies 548 million ha (or 38% of the degraded terrain).

2.1.1.2 Compaction, sealing and crusting

Compaction is by far the most important subtype of physical soil deterioration which occupies 68 million ha worldwide. Compaction is usually caused by the use of heavy machinery on soils with low structural stability. Sealing and crusting of the topsoil occurs in particular if the soil cover does not provide sufficient protection to the impact of raindrops. Soils low in organic matter content with poorly sorted sand fractions and appreciable amounts of silt are particularly vulnerable. Both compaction and crusting can be caused by cattle trampling. Compaction and crusting will make tillage more costly, impede or delay seedling emergence, and lead to a decrease in water infiltration capacity, causing in its turn a higher surface run-off, which may lead to significant water erosion.

2.1.1.3 Water logging

Water logging is caused by a rise in groundwater close to the soil surface or inadequate drainage of surface water, often resulting from poor irrigation management. As a result of water logging, water saturates the root zone of plants, leading to oxygen deficiency. It is also caused by flooding by river water and submergence by rain water caused by human intervention in natural drainage systems.

2.1.1.4 Chemical degradation

Loss of organic matter

Soil organic carbon (SOC) is of global importance because of its role in the global carbon cycle and therefore, the part it plays in the mitigation or worsening of atmospheric levels of greenhouse gases. It is also important on local scale as it

determines ecosystem and agro-ecosystem function, influencing soil fertility, water holding capacity and many other functions. Loss of nutrients and/or organic matter occurs if agriculture is practiced on poor or moderately fertile soils without sufficient application of manure or fertiliser. This causes a general depletion of the soils and leads to decreased production.

Anthropogenic activities such as cultivation and deforestation and other land use types (Gong et al. 2006) have also been found to rapidly decrease carbon and nitrogen in virgin soils as Saikh et al. (1998) found in a case study in India. Lobe et al. (2001) found that prolonged arable cropping from sandy soils of the South African Highveld reduced soil C and N concentrations by 65 and 55% respectively over a period of 98 years. Other long term South African studies (Woomer 1993; Dominy et al. 2002; Mills and Fey 2003; Mills and Fey 2004; Snyman and du Preez 2005) and studies in other parts of the world (Schulten 1995; Schnitzer 2000; Yang et al. 2003; Zhang et al. 2004; Turner et al. 2005; GEFSOC 2006) have well documented the effect of the decline in soil organic carbon following prolonged cultivation and the detrimental effects of decreased SOC.

Activities such as mining, in addition to the general soil degradation they cause, have a profound effect on soil carbon dynamics. During opencast coal mining for example, the removal of earth surface produces overburden materials and its pilling on the unmined land creates overburden dumps. These dumps are physically, nutritionally and biologically poor in nature (Cornwell and Stone 1968; Wali 1987; Hüttl 1997; Zier et al. 1999; Lin et al. 2005). A recent comparative study between mine rehabilitated soil and natural soil in Namaqualand, south west of South Africa, revealed that mining operations result in the formation of saline sand tailings, stripped of a large portion of clay and organic matter fraction (Prinsloo 2005). The natural succession on these lands also takes longer.

Salinisation

Soil degradation from salt accumulation, sodication, or both, is a threat in many irrigated lands (Herrero and Perez-Coveta 2005). Saline soils are those soils with high amounts of soluble salts including soil salts such as sulphates (SO_4^{2-}), carbonates (CO_3^{2-}) and chlorides (Cl^-). These soils often exhibit a whitish surface crust when dry. Sodic soils are those soils with high amounts of sodium (Na^+) where soil conditions can restrict plant root growth and can make tillage difficult (McWilliams 2003).

Natural salinisation occurs as a result of the weathering of rocks by primarily physical but also chemical and biological processes. However, this process of natural salinisation is being exacerbated by anthropogenic influences. According to Oldeman et al. (1991), human-induced salinisation can be the result of the following three main causes:

i. Poor management of irrigation schemes. A high salt content of the irrigation water or too little attention given to the drainage of irrigated fields can easily lead to a rapid salinisation of the soils. This type of salinisation mainly occurs under (semi-) arid conditions and covers small areas.
ii. Intruding of seawater or a fossil saline ground water body into ground water reserves of good quality. This sometimes happens in coastal regions with an excessive use of ground water but can also occur in (closed) basins with aquifers of different salt contents.
iii. Evapo-transpiration of soil moisture in soils on salt-containing parent material or with saline ground water as a result of an increase in human activities.

High soil salinity (high EC) causes plant cell dehydration, reduced plant growth and possibly death in less tolerant plants.

Acidification

Acidification occurs in coastal regions upon drainage of pyrite-containing soils. As a result, pyrite will oxidize to, among others, sulphuric acid, which strongly reduces the agricultural potential of the soils because of extremely low pH values. Another type of acidification is caused by over-application of acidifying fertiliser which may also lead to

strong acidification and reduced agricultural potential. Soil acidification is also induced by mining activities which cause oxidation of metal sulphides contained in the mine spoils (Thomas 1996; Hüttl 1997; Chabbi and Rumpel 2004; Lin et al. 2005). This reduces natural colonization of vegetation in the mine site which in turn may result in severe soil erosion. Acid mine drainage from the mine site can cause the degradation of the downstream aquatic ecosystems due to the low pH (Lin et al. 2005).

2.1.1.5 Biological degradation

Many key soil functions are underpinned by biodiversity and organic matter. Organic matter enters soils principally from plant remains and organic manure additions, and is primarily made up of carbon, nitrogen and phosphorus. Biodiversity and organic matter can decline due to erosion or pollution, leading to a reduction in soil functions such as control of water and gas flows. The four major indications of soil biological degradation as identified by Sims (1990) are:

i. Loss of community diversity and which is indicated by sub-optimum proportions of different species of soil micro-organisms which leads to the disturbance of many key soil functions underpinned by biodiversity and organic matter.
ii. The disturbance in nutrient cycling which is indicated by the decrease in soil enzymes, various components of the N cycle, cellulose or wood degradation, and of respiration.
iii. Accumulation of pollutants: The soil's ability to dechlorinate organic compounds is impaired especially in sulphate-rich anaerobic environments. Heavy application of animal wastes to low- pH soils can lead to build-up of ammonium and a concomitant reduced functioning of *Nitrobacter*. This effect can result in nitrite accumulation.
iv. Change in the redox status of the soil which is indicated by the development of anaerobic conditions as a result of compaction or water logging. Oxygen diffusion is only 1/10,000 as fast through water-filled soil pores as it is through

air-filled pores. In addition, reducing conditions result in the production of large amounts of methane which are associated with anoxia.

2.1.2 Drought

Drought is a climatic event that frequently occurs in arid, semi-arid and dry sub-humid lands. The World Meteorological Organization (WMO) defines drought as "a deficit of rainfall in respect to long term mean, affecting a large area for one or several seasons or years that drastically reduces primary production in natural ecosystems and rain fed agriculture" (WMO 1975). Rainfall deficit is the most important climatic variable indicating the presence of severe drought. Rainfall shortages cause a decrease in water supply to levels that are insufficient to fulfil requirements for domestic, agricultural, and ecological demands. Impacts of drought are evident in the state of soil and vegetation and are the most severe in landscapes that have been destabilised by anthropogenic pressure.

The distinction between human induced droughts and those caused by natural climatic conditions becomes all more important, given the wide spread concern about likelihood of human induced global climate change (Glantz 1994). Drought is also a regular phenomenon in South Africa. Impacts of the 1991-92 drought have included severe agricultural losses to commercial and subsistence farmers, reduction in reservoir levels and have exacerbated the plight of rural communities. It has been argued that the severity of drought impacts has been more a consequence of mishandling of drought situations, farm management and agricultural systems in South Africa than a consequence of the reduction in rainfall (Vogel 1994). Other countries share the same experience as South Africa.

The Sudano Sahelian zone located between latitudes 10° N and 20° N and extending from the Cape Verde in the West to Somalia in the East is a good example of a region severely affected by drought. The area has received much international attention and is said to be undergoing severe desertification, which among other factors, is attributed to prolonged drought (Obia 1997). Initially, drought in this area was considered to be a

natural phenomenon. But closer scrutiny of the facts revealed that human activities (including policies) had unwittingly made the bad consequences of drought even worse. In actual fact, the history of human occupation of drylands appears to be punctuated with numerous examples of productive land being lost to desert, either through mismanagement, over use of the environment or through natural changes in the environment itself or a combination of the two (Glantz 1994; Thomas and Middleton 1994). Clearly, the mix of these activities in marginal areas has led to widespread degradation and desertification, not only in South Africa and the Sahel region but also in other parts of the world. The following overview of desertification is important in attempting to understand the implications of drought.

2.1.3 Desertification

Desertification means land degradation in arid, semi-arid and dry sub-humid areas resulting from various factors, including climatic variations and human activities (UN 1994; Eswaran et al. 2001). Desertification involves the loss of biological or economic productivity and complexity in croplands, pastures and woodlands. Seventy percent of the worlds dry lands (excluding hyper-arid deserts), or some 3600 million hectares, are degraded (UNCCD 2005). Desertification arises from the interaction between a difficult, unreliable (mainly to climatic variability) and sensitive dryland environment and man's use and occupation of it in his effort to make a living (UNCCD 1977). The most cited forms of unsustainable land use are over-cultivation, over-grazing, deforestation and poor irrigation practices. While drought is often associated with land degradation, it is a natural phenomenon that occurs when rainfall is significantly below normal recorded levels for a long time. In addition, other non-climatic variables that contribute to desertification include soil structure and texture, topography and vegetation types that are characteristic of an area.

2.2 Land degradation at a global scale

There are 15.9 billion hectares (ha) of land on earth, of which 4.1 billion hectares (21%) are covered by forest land, 3.1 billion ha (19.1%) by grassland, 1.5 billion ha (9.4%) by arable land, and the remainder (50.5%) by ice, desert, stone and mountain terrain

(Wynberg 1993). In 1980, it was projected that close to one third of the worlds arable portion of land on earth would be destroyed by the end of the last century. Similarly, the remaining area of unlogged productive tropical forest would be halved (IUCN 1980).

According to UNEP (1997), land degradation is one of the world's major socio-economic and environmental problems affecting one billion people in 110 countries worldwide and is prevalent across about 40 per cent of the Earth's surface. Land degradation is a global environmental problem about which a great deal has been written in the past 50 years or so and has attracted much popular attention. Almost one half of the world's land surface is classified as 'dry land', comprised of hyper arid, arid, semi-arid and dry sub humid areas, and a substantial proportion of this is thought to be in various degrees of degradation. Global assessment of these areas suggests that degradation in these areas impact on more than 1 billion people who are dependent on the land for their livelihoods.

2.3 Land degradation in South Africa

South Africa's land degradation problems have their roots on environmental and social factors (Meadows and Hoffman 2002). South Africa is subject to a high degree of variance with respect to certain climatic elements that are fundamental to the economic utilization of the land. It is semi-arid and experiences catastrophic droughts. In general, Southern Africa is fundamentally an arid subcontinent, more especially in the interior and western parts, so that most ecological – and, for that matter, economic – processes are, to a greater or lesser extent, limited by water availability (Meadows 2006).

South Africa, receives an average annual rainfall of 498 mm, however, over 60% of the country receives less than 500 mm per year which is academically the minimum for successful dryland cropping and 21% less than 200 mm per year (de Villiers et al. 2004). Global climate change is compounding the situation even further by threatening to worsen desertification in some parts of the country, making it even more difficult to feed a rapidly growing population.

Another peculiarity of South Africa's soil base is that 8% of the country's arable land, comprising 51.6% of prime agricultural land, is underlain by exploitable coal deposits. Besides coal, 18 other economically important minerals are being mined using opencast methods. This while reducing the already limited amount of arable land on the one hand, contributes to the destruction of the land which is accompanied also by pollution.

Against this backdrop of aridity and climatic variability, which result in the susceptibility of the land to degradation lies a uniquely South African land tenure policy rooted in its colonial and apartheid past. It is well known that most of the areas of South Africa managed under communal land tenure system, approximately 14% of the country, are those associated with historically black 'homelands' of the apartheid regime. Such areas have been significantly under resourced compared to the white agricultural land owned and managed under the commercial farming system. It is therefore a reasonable hypothesis that there are substantial differences in the degradation status of communal and commercial areas arising out of differential access to economic and political power. Communal areas have high populations, high stocking rates which is partly due to less intensification of agriculture due to the dependency of most homeland economies on migrant labour onto the mines and as such, these areas are arguably more susceptible to degradation than commercial areas.

Hoffman (2000) estimates soil degradation alone to cost South Africa nearly R2 billion per annum. Significant costs are incurred because of erosion of arable lands. It has been quoted in many publications that South Africa loses approximately 300 to 400 million tons of soil to soil erosion on an annual basis. National assessments on the nature and extent of soil erosion have pointed to the wide spread occurrence of rill and gully erosion which result from the soil being exposed to the direct impact of precipitation and subsequent run off as a result of grazing pressure and, in the case of arable agriculture, due to crop rotation and fallow periods. Erosion generally leads to siltation of dams and increased costs of water purification. The overall cost of land degradation is however, much higher than the quoted figure, as it includes other problems such as loss of plant cover, alien plants, bush encroachment and deforestation.

The political movement of South Africa from 'separate development' policies (as well the end of international isolation) into democracy has also changed the approach in trying to address land degradation. In January 1995, South Africa signed an international agreement; the United Nations Convention to Combat Desertification. South Africa has as results of this committed itself through the Department of Environmental Affairs and Tourism (DEA&T) to a National Action Programme (NAP) to combat degradation. Through NAP, Hoffman (1999) collected through research census figures, scientific literature and information provided by agricultural extension officers and conservation technicians from the Department of Agriculture which were published on the National Botanical Institute (NBI) website (www.nbi.ac.za/landdeg).

2.4 Conclusion

The above discussion sought to outline the problem of land degradation, the key aspects responsible for it and finally, the situation in South Africa as well as at a global scale. The discussion above bears a lot of significance, especially that this is a global problem. Central to this problem is the loss of soil organic matter which is of great importance for soil development and plant growth. The various ways by which soil organic matter is lost have been alluded to in the above discussion. In order to reduce soil degradation, particularly soil erosion and to initialize soil recovery, numerous activities in soil rehabilitation and restoration have been undertaken in many parts of the world. These activities are supported by international organisations such as the UN which has released a Convention to Combat Desertification and its subsequent modifications (UNCCD 1994; UNCCD 2005a; UNCCD 2005b). The success of these activities can only be achieved if they can improve soil conditions in a sustainable way for the establishment of both micro- and macroflora. In order for this to take place there should be a sufficient supply of nutrients as well as microbiologically degradable soil organic matter in the soil.

Soil organic matter is any material in the soil that was originally produced by living organisms. At any given time it consists of a range of materials varying from the intact

original tissues of plants (mainly) and animals to the substantially decomposed mixture of materials known as humus. The original tissues contain a wide range of organic compounds which typically decompose at different rates. In a soil which at first has no readily decomposable materials, adding fresh tissue under favourable conditions immediately starts rapid multiplication of bacteria, fungi, and actinomycetes, which are soon actively decomposing the fresh tissue. As the most readily available energy sources (carbohydrates, fats and proteins) are used up, those microorganisms again become relatively inactive, leaving behind a dark mixture usually referred to as humus. Newly-formed humus is a combination of resistant materials from the original plant tissue and compounds synthesized as part of the microorganisms' tissue which remain as the organisms die. It is quite resistant to further microbial attack, so its nitrogen and other essential nutrients are protected from ready solubility and dissipation. Humus holds water and minerals extremely well; it sticks together very well, so helps soil establish and maintain a strong crumb structure; it provides some nutrients as it is slowly decayed by microbial activity (Dunn 1994).

In general, the quality of the soil organic matter is of great importance for soil development and plant growth. Soil organic matter plays an important role in sustaining soil fertility, and hence in sustainable agricultural production. In addition to being a source of plant nutrient, it improves the physico-chemical and biological properties of the soil. As a result of these improvements, the soil:

i. Becomes more resistant to stresses such as drought, diseases and toxicity;
ii. Helps the crop in improved uptake of plant nutrients; and potassium and phosphorus.
iii. Possesses an active nutrient cycling capacity because of vigorous microbial activity.

These advantages manifest themselves in reduced cropping risks, higher yields and lower outlays on inorganic fertilisers for farmers. However, the natural formation of the stable humus fractions in impoverished soil is a slow process and therefore needs to be supplemented with high-grade humic substances.

One possible way in which this could be achieved, would be the use of geogenic resources (e.g. peat) or humus precursors like composts or sterilized sewage sludge, which can accelerate soil improvement. However these are problematic because their compositions frequently vary and the pollutant content and composition often exceeds the specifications laid down by soil protection acts or laws which regulate the use of such materials. Mineral fertilisers with high nitrogen contents have often been used as well but they often have led to serious ground water pollution and the presence of nitrates in vegetables. This situation has been even more serious in places where the soil is permeable and thereby quickly allowing agricultural chemicals to permeate into the soil to the ground water level.

In light of the foregoing problems, it is necessary to find technical solutions to accelerate the natural humification process using modified appropriate organic starting materials. Organic based fertilisers with the required composition and properties could be used for this process. Amongst the most promising organic starting materials are technical lignins (and perhaps lignite where it is available) obtainable in huge supply from the pulping industry. Although native lignin is the second most abundant, renewable polymeric component of the biomass after cellulose, only 5% has been utilised in applications other than energy production in pulp mills. Based on pulp and paper production statistics, about 50×10^6 tons of lignin are produced from woody plants at pulp mills worldwide each year (Wünning 2001). Among the 5% used in application other than energy production, are lignosulphonates. Lignosulphonates find applications mainly as binders for animal feed pellets, in bricks, ceramics, and road stabilisers, as dispersants for oil well drilling products, dyestuffs, pesticides, carbon black, water treatment additives etc. The use of soil improving products derived from technical lignins could provide a new approach to maintain an ecological balance by returning an industrial by-product (technical lignin) or redistributing a natural product (brown coal) to the natural bio cycle.

The following discussion alludes more on the use of different fertilizing materials including inorganic fertilisers, organic materials such as manures, compost, sewage

sludge and peat and their effect on the environment. The discussion commences with the description of organic matter and its role in supporting soil functions.

2.5 Land rehabilitation: Nutrient cycling and sustainable development

2.5.1 Nitrogen fertilisers (N-fertilisers)

N-fertilisers are widely used in agricultural practices in different organic and inorganic forms to enhance crop productivity. They are most commonly produced by binding atmospheric nitrogen (N_2); i.e., converting it into a form that can be utilized by plants using various methods including ammonium synthesis (*Haber-Bosch* process), calcium cyanide synthesis; nitrate synthesis (arc-gap process). The ammonia synthesis is quantitatively the most important method of producing N-fertilisers, with ammonia being further processed into various N-fertilisers, for example, limestone ammonium nitrate or urea (Finck 1982).

The effectiveness of any fertiliser depends mainly on maintaining sufficient concentration of nitrogen within the plant root zone for a desired period of time. However, due to processes such as degradation (chemical, photo-chemical and biological), vitalisation, leaching, adsorption and immobilization, the effective compound often diminishes too quickly. As a consequence, soil application fertilisers, including N-fertilisers, require relatively large dosages to be applied to fulfil this requirement. This however, may cause environmental pollution through leaching of the excess nitrogen in the form of nitrates into ground water and a high concentration of nitrates in plants (Hadas et al. 1999; Costa et al. 2002).

The leaching of nitrates from fertilisers is of great environmental concern from the health and environmental point of views as high nitrate contents in plants and water could result in disease in case of human consumption. Widely known examples of diseases caused by nitrogen pollution include:

i. the decline in the oxygen conveying capacity of blood under the effect of nitrates after the reduction to nitrites, when ingested (through drinking water or formula milk prepared from water high in nitrate concentration) by infants

(infants under the age of six months are more at risk). The condition is called "methomeglobinemia" or "blue baby syndrome"[1] (Majumdar 2003)

ii. gastric cancer in adults for which a possible link with nitrite or nitrosamines has been suggested (Correa et al. 1975; Walters 1983; Forman et al. 1985; Dutt et al. 1987; Giacosa et al. 1988; Packer et al. 1988; Siddiqi et al. 1992; van Loona et al. 1997);

iii. other diseases such as goitre (Ubom 1991), birth defects (Schubert et al. 1997). Nitrates are also associated with brain cancer (Forman 2004) and

iv. eutrophication of surface waters (Jickells 2005).

From a South African perspective, ground water is often cited as the cheapest form of water supply to previously unserved communities. Holtzhausen (2005) reports that rural villages and small towns from the Northern Cape to the far southeast of Limpopo (see figure 2-2) are severely affected by high nitrate concentrations in ground water. Concentrations range from 50 – 100 mg/l and even well above 100 mg/l in some areas while in the southern Kalahari concentrations of up to 500 mg/l of nitrate occur in certain areas. HIV/Aids is increasing infants' risk of ingesting high nitrate water as HIV positive mothers are advised to refrain from breast-feeding their babies and rather bottle-feeding them. Thus the pandemic has introduced an urgency element for groundwater nitrate control measures in our region.

[1] Nitrate itself is not toxic to humans. Nitrate becomes a problem only when it is converted to nitrite in the human body resulting in methomeglobinemia. Nitrite ions (NO_2^-) in blood can inactivate hemoglobin with dangerous consequences. The inactivation occurs because nitrite ions change hemoglobin whose iron is doubly-charged (Fe^{++}) and can carry oxygen into methemoglobin, whose iron is triply-charged iron (Fe^{+++}) and cannot carry oxygen. Socolow, H.R. (1999). "Nitrogen management and the future of food: Lessons from the management of energy and carbon." Proc. Natl. Acad. Sci. USA **96**: 6001–6008.

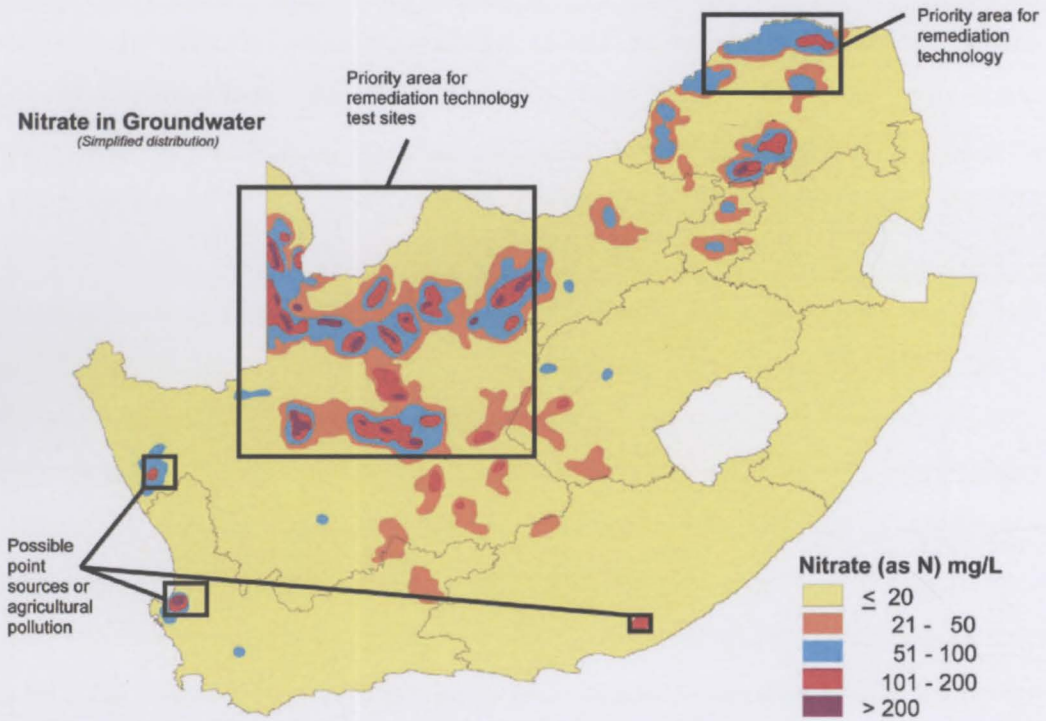

Figure 2-2: A simplified distribution of areas in which ground water is polluted by nitrates (Holtzhausen 2005).

The rehabilitation of polluted aquifers is a tedious, expensive and time-consuming operation with limited success. While efficient methods exist to treat nitrate-rich groundwater, these require high technology, are expensive or both. Present treatment methods range from biological denitrification to ion exchange and reverse osmosis although *in-situ* methods (i.e. treating the water within the aquifer) are increasingly being researched. The cost implications make prevention of the problem all the more important. Although the development of natural soil nitrogen cannot be controlled, anthropogenic nitrogen inputs are manageable and hence most groundwater nitrate problems can be avoided by reducing releases into the soil and subsurface environment (Holtzhausen 2005).

N-fertilisers continue to play a decisive role in food production, and this irrespective of which technologies are applied. It is estimated that globally roughly 40% (37% to 43%) of the world's dietary protein supply in the mid-1990s originated in synthetic nitrogen in the form of N-fertilisers (FAO 2000). At the same time, human demand of these

resources is growing fast, coupled with the growth in population numbers. However, due to the environmental and health concerns listed above efforts are being made to curb over application of these materials and hence the reduction of leaching of nitrates into ground water.

Standards for nitrate concentration in potable water have been set at 50mg NO_3^- N/litre in the United States (USEPA 1995) 10mg and per litre in South Africa and the European Community (EC 1998). In order for these regulations to be maintained, it is essential that the specific nitrogen needs of individual species of cultivated plants be determined on the one hand and the development of the method of applying the substance in order to maintain nutrient availability on the other hand (Lang 1996). In addition, the integration of knowledge related to environmental conditions of certain areas with the soil, water and crop management practices simultaneously help prevent unfavourable processes leading to nitrate leaching, thus water resources may be partially protected from leaching of agricultural origin (Nemeth 1995).

2.5.2 Controlled nitrogen release fertilisers

One of the solutions suggested to cope with the problem of nitrogen leaching into ground water from N-fertilisers is the incorporation of the fertilisers in controlled-release formulations. The potential benefits of following this procedure as discussed by Allen (1984) include:

i. a more efficient use of N by the crop
ii. less leaching of nitrates into ground water
iii. lower toxicity as excessive nutrient supply, commonly result from an application of conventional soluble fertilisers
iv. high concentration of salts in the root zone. This may induce osmotic stress and cause injuries to plants during different stages of growth or undesired development such as lodging
v. longer lasting N supply
vi. reduced vitalisation losses of N, and

vii. lower application costs.

The terms controlled release fertiliser (CRF) and slow release fertiliser (SRF) are often used interchangeably. However, CRF is more acceptable when the factors dominating the rate, pattern and the duration are well known and controllable during CRF preparation. SRF involve the release of nitrogen in a slow manner than common fertilisers. However, the rate, the pattern and the duration of release are not well understood (Shaviv 2001).

CRFs or SRFs are generally classified into (Allen 1974; Shaviv 2001):

i. Biodegradable organic compounds of low-solubility;
ii. Fertilisers in which a physical barrier controls the release i.e. granules coated by hydrophobic polymers or matrices in which the soluble active substance is dispersed in a continuum that restricts the solubility of a fertiliser and;
iii. Inorganic low solubility compounds i.e. fertilisers such as metal phosphates (e.g. Mg NH_4PO_4).

The chemistry of a selected number CRFs or SRFs and their mode of action in the soil has been reviewed by Allen (1984). Comparative studies on the effects of CRFs or SRFs on the growth of various crops have also been carried out (Sartain 1984; Mikkelsen et al. 1994). It is from these and other studies that it has been found that controlled nitrogen release fertilisers offer more benefits in promoting plant growth and reducing leaching of nitrogen into the environment.

Some studies on the behaviour of CRFs/SRFs have focussed on the modelling of the release of nutrients from these materials in order to further understand their behaviour (Friedman and Mualem 1994; Kochba et al. 1994; Al-Zahrani 1999; Wolf et al. 2005). Shaviv (2001) has also discussed several models which can be used in order to monitor nutrient release rate. This is particularly important as these models can be used to

analyze field data or plan experiments well with CRFs and SRFs provided that reliable knowledge exists regarding a specific crop.

The use of controlled – release fertilisers is starting to evolve as a promising direction, offering an excellent means to improve management of nutrient application and by this reducing significantly environmental threats while maintaining high crop yields of good quality. The total amount of SRFs/CRFs consumed worldwide in 1996/1997 was about 560 000 tons. The largest consumption is in the United States and Canada, where about 70% of total amount is used. In Japan, most of the CRFs are used in agriculture, mainly for growing fruits, vegetables and rice and only a small percentage is used for turf and ornamental agriculture. In the United States, Canada and Europe, about 90% of the total consumption is used for non-farm purposes (golf courses, nurseries, professional lawns, landscaping); only about 10% is used in agriculture mainly for vegetables, melons, strawberries, citrus and other fruits (Shaviv 2001).

The cost effectiveness and limited recognition of the potential benefits to be gained from CRFs are so far the main reason for their limited consumption. In addition, one reservation regarding the use of SRF/CRFs for reducing environmental problems related to fertiliser production is the extent to which the various materials used for preparing SRF/CRFs (plastics, formaldehydes, sulphur, etc.) contributes to environmental pollution. Hence new approaches have been investigated using materials from different sources to manufacture fertilisers in a more environmentally friendly way.

2.5.3 Organic waste products

The use of organic wastes such as sewage sludge, composts, manure, peat, manure etc., have always been seen as an attractive proposition for turning organic waste materials into agricultural resources. However, these materials are sometimes also associated with environmental pollution. Some of them besides being sources of environmental pollution through nitrates are also sources of pathogens. A short survey of the possible benefits that could be derived from use of these materials as well as potential

environmental problems, especially which could arise from the application of these materials, is presented below.

2.5.3.1 Sewage sludge

Sewage sludge is an inevitable end product of waste water treatment presented as a concentrate of waste material. Society is faced with a major challenge of disposing it as landfill and incineration are currently the major forms of disposal and only a small part goes into agriculture. This therefore, provides an opportunity for beneficial usage of this resource by creating a cycle whereby sludge derived from agricultural activity can be returned to soil and thereby creating a sustainable and ecologically sound management of this material (Langenkamp et al. 2001). Sewage sludge is rich in organic matter and nutrients and hence can be successfully used in agricultural practices especially in arid countries such as South Africa (Hoffman 2000). It can be incorporated into soil as a soil conditioner and hence improving the physical, chemical and biological properties of soils which may enhance crop growth. It may be a useful source of trace elements to plants if the concentrations of these elements are low in unamended soil (Rate et al. 2004).

Sources of pollution

While these benefits can be exploited with treated sludge, untreated sludge poses a lot of problems to the environment as a result of the multitudes of potentially toxic compounds which it contains including among others chlorinated hydrocarbons, polynuclear hydrocarbons, nitrates and heavy metals. In South Africa, sewage sludge drying beds, land application of sludge and irrigation of partly treated wastewater are some of the causes of point source pollution responsible for excessive nitrate levels in ground water especially around urban centres (Holtzhausen 2005).

Heavy metals on the other hand can be retained in the soil when sewage sludge is applied and can accumulate to a point whereby they are toxic when taken up by plants. Thus, due to uptake by crops, heavy metals can be toxic to human and animals as well. They can have long lasting effects on biological functions of the soil. The heavy metal

of major concern because of its possible toxicity and danger to the human food chain is cadmium (Cd). Other heavy metals of importance are copper (Cu), nickel (Ni) and Zinc (Zn). Organic compounds such as pesticides, polychlorinated biphenyls, halogenated aliphatics, ethers and aromatic hydrocarbons are products of industrial waste water which could end up in waste water sludges.

In addition to pollutants of chemical nature, sewage sludges also contain a wide variety of pathogens which can be infectious to different species of animals and plants as well as humans. The four major types of human pathogenic organisms namely bacteria, viruses, protozoa and helminths may all be present in sewage sludges. The origins of these pathogens and the danger they pose have been discussed by Chale-Matsau (2005).

Socio-economic issues

There are also socio-economic issues regarding the usage of sewage sludge. According to the NRC (1996) and Chale-Matsau (2005), farmers and the food industry have concerns that the use of untreated sludge can affect the safety of food products, sustainability of agricultural land and may carry economic and liability risks. There are also concerns that the use of contaminated sludge could negatively affect the export market. For fear of food borne diseases, some countries may refuse importation of food produced under such conditions.

In many African countries, especially South Africa (and perhaps other parts of the world), a large percentage of the population lives in poverty. It can be expected that these households can be easily affected by contaminated crops. The high incidence of HIV/AIDS (Human Immunodeficiency Virus/ Acquired Immuno Deficiency Syndrome) infection in the country could also result into more pathogenic infections if such communities are exposed to contaminated crops.

Dealing with the problem

To ensure the re-use of sludge as fertiliser without jeopardizing human health, it must fulfil the public and legislative requirements. In this regard, the Water Research

Commission of South Africa has published a legislative guide for permissible utilization and disposal of sewage sludge (with the same title) to be used by treatment plant operators, water practitioners, engineers, farmers and health workers alike, to help in dealing and handling this material (WRC 1997). The guide is based on current national and international research data and regulatory strategies such as those published by the US Environmental Protection Agency (USEPA 1995), the European Commission Joint Research Centre Institute for Environment and Sustainability Soil and Waste Unit (Langenkamp et al. 2001).

2.5.3.2 Livestock manure

The application of animal manures in agriculture like sewage sludge provides an alternative for treatment and disposal of these materials. Animal manures are used to improve the fertility of the soil by supplying plant macro nutrients such as nitrogen, phosphorus, potassium as well as micronutrients. In addition to the supply of nutrients, manure generally improves soil tilth, aeration, and water-holding capacity of the soil and promotes growth of beneficial soil organisms.

Sources of pollution

While well-applied manure has numerous positive attributes, excessive amounts of manure have generated considerable concern over its potential to impair water quality. The major cause for excessive application of manure is the variation in the nutritional content which is dependent on animal species, age, ration and feed consumption as well as with different methods of storage, treatment and land application. Extensive data is available on manure nutrients "as produced" by animals and poultry. However, this data is of limited use because of variations introduced by the different manure management methods (Fulhage 2000). Such variations cause difficulties in applying precise and appropriate amounts as per crop requirement. In addition, roughly half of the nitrogen in manure is unavailable to crops and therefore results in farmers applying nearly twice the amount of total nitrogen than would otherwise be needed. Poultry manure for example has a high P content which can be detrimental to plants and induces

micronutrient deficiencies if excessive quantities are applied. These factors increase the amount of polluted runoff coming from manure applied to cropland.

Environmental concerns with manure are not only limited to soil application but also the storage and handling of animal waste and related agricultural practices are some of the main contributing factors to groundwater contamination (Fleming 1999). Animal manures contain nutrients particularly N and P, dissolved mineral salts, toxic metals, microorganisms and antibiotics. Among these constituents, nitrates, ammonia, and potentially pathogenic organisms, hormones derived or excreted by animal waste and carcasses are the most common groundwater pollutants (USEPA 2004). In addition, the nature of modern animal agriculture with its highly concentrated production facilities and reliance upon feed supplements to maintain animal health and productivity, has also raised serious questions about the effects of animal manure on the quality of soil, water, atmosphere and food supply (LPES 2006).

With regards to pathogens, Hutchison et al. (2000) report on some of the key areas which pose a high risk in terms of pathogen transfer from animal manures into the food chain i.e.:

i. Where animal manures are removed from animal housing and immediately applied to land.
ii. Where manures originate from young livestock and animals which have just given birth.
iii. Where manures are bought in from another farm with an unknown disease history.
iv. Manures from livestock on farms with poor disease control.
v. Land application of manures during cool, wet weather because of increased survival and transfer by surface runoff.
vi. Management of manures where the relevant Codes of Practice are not being followed e.g. non-adherence to no-graze periods.
vii. Growing food crops on land recently used for rearing livestock or where manures have been applied recently.

viii. Livestock grazing where pathogens in fresh excreta are likely to be transferred onto fodder.

ix. Stock drinking from water contaminated with animal faeces.

x. Use of contaminated irrigation water.

Dealing with the problem

Animal manures can only be better beneficiated if they are analysed prior to application and proper attention is given to their composition and decisions on rates, timing and placement are made correspondingly. This should lead to a drastic reduction of mineral fertiliser use, nutrient surpluses and environmental pollution and enhance the nitrogen fertiliser value of manure (Schroder 2005). In addition, stream pollution from animal manures can be avoided by adopting simple principles such as the maintenance of separation distances and buffer areas between land used for manure application and surface water drainage (Mancl and Veenhuizen 2006).

2.5.3.3 Compost

The development of agricultural systems in some countries has left a legacy of high concentrations of animal wastes (often in liquid form and with low solids content and, therefore, not amenable to composting) and high levels of carbon (straw) residues in others. As a result of such scenarios, agricultural wastes have become a waste disposal problem and a potential threat to the environment through BOD levels, ground water contamination, ammonia emissions, odours and pathogenic loadings.

Composting in general, has received increasing interest as a method for handling various types of animal manures (DeLaune et al. 2004), sewage sludge as well as agricultural wastes. Organic wastes are diverted from landfill or other disposal sites. Composting agricultural wastes offers benefits such as enhanced soil fertility and soil health that engender increased agricultural productivity, improved soil biodiversity, reduced ecological risks and a better environment (FAO 2003). The use of compost on low organic matter soils results in improved moisture and nutrient retention, decreased soil erosion, reduced surface crusting, suppression of plant diseases and improved soil

tilth. Composting of organic wastes kills weed seeds, pathogenic bacteria and viruses and stabilizes some of the nutrients in wastes so that they are not as readily leached out. This decreases the potential for ground and surface water contamination (Anonymous 1996). The biodegradation that occurs during composting allows more SO_2 to dissolve. This makes the material more desirable by improving physiological and biological conditions, including improved aeration, greater ease of seedbed preparation, improved water holding capacity and stimulation of soil microorganism activity (Henry and White 1993). Composting also provides a method of dealing with agricultural waste, which is the largest component of waste produced by society.

From a nutrient supply perspective, the major problem associated with use of compost is the difficulty in predicting N supply from them. This mainly stems from the reduction in inorganic N content during composting through immobilization as organic N together with volatilization or leaching of N which often reduces inorganic N levels in compost to less than 15% of the total N (Lynch et al. 2004). This mainly occurs under aerobic conditions as the aerobic microbes decompose the organic matter in the compost and release the nitrogen to the atmosphere. In this respect, the only way to prevent this loss of nitrogen is to store the organic matter under anaerobic conditions. However, under these anaerobic conditions the carbon is lost as methane but nitrogen, phosphorus, and potassium nutrients are conserved.

Due to the cost and N loss associated with composting especially poultry litter, composting is not economical from an agronomic perspective and hence the use of alum and H_3PO_4 to reduce NH_3 volatilization (DeLaune et al. 2004). There is also the problem of the unfavourable N to P ratio in animal wastes and most compost relative to crop N and P uptake. This leads to agronomically excessive levels of soil P if composts are used over a long term for N crop requirements. Hence, knowledge of its relative nutrient supply capacity needs to be established prior to use to give planners the ability to supplement with other N-containing materials to optimize yields (Wolkowski 2003). These factors have resulted in limited routine use of composts and have led to recommendations that they be considered solely as soil amendments in crop production.

From engineering and commercial aspects, composting municipal and industrial biosolids for example can present unique problems because the organics may still contain 70 to 80% water. The presence of so much water can result in reduced composting temperatures and inefficient operation if moisture is not controlled. The high moisture content, lack of porosity, tendency to compact, and the need to dry dewatered sludge during composting makes biosolids composting somewhat unique and often difficult. An understanding of the thermodynamics of composting is essential for designing workable sludge composting systems (Haug 1993).

Dry substrates present their own unique blend of challenges. Dry substrates such as agricultural wastes and particularly refuse and yard wastes are heterogeneous and may require source separation as well as particle size reduction to improve the rate of composting. These wastes often lack sufficient nutrients, particularly nitrogen, which may have to be added to avoid kinetic rate limitations.

2.5.3.4 Summary

In summary, it is acknowledged that the recycling of resources is a pillar of sustainable development. It is also acknowledged that the recycling of sewage sludge as fertiliser could decrease the need for commercial fertiliser. In addition, substantial amounts of energy are used for the collection and treatment of sewage and energy is also produced in the treatment process. Recovering some of it is in line with sustainable development. This can be done via biogas or heat pumps which is not yet practiced in South Africa (Morrison et al. 2001). However, the pollution it brings about should also be weighed against the benefits.

2.6 Lignin biomass as a raw material for humus products

The desire to use lignin as a plant nutrient is prompted by two aspects namely:

i. The chemical relationship between lignin and humus i.e. lignin is an important precursor of humus or soil organic matter (SOM) in nature. Humic substances

are required for soil fertility and they influence the structure and biological activities of the soil.

ii. The availability of large quantities of technical lignins as by-products from the pulping industry as well as other chemical processes of wood utilization.

Over the past few decades, the amount of chemical pulp has increased globally, and so is the amount of technical lignins (the by-products of the pulping process). In modern industrial chemical pulping methods, technical lignins are used for the generation of thermal energy for steam generation. In addition, they form an important component of the chemical recovery system.

Efforts directed at lignin beneficiation continue to grow especially that lignin is sustainable renewable material. However, prior to utilisation of lignin, the following needs to be taken into consideration:

i. The methods used in the generation of pulp degrade the lignin. As such lignin is always obtained in an altered form which differs from the natural lignin (protolignin) chemically in terms of functional groups, molecular mass and molecular mass distribution, as well as the number of C-C and C-O bonds.

ii. Technical lignins form only a component (major component) of spent liquors from the pulping process. These liquors contain a number of substances of which the organic component partly consists of low molecular weight substances from the degradation of the wood or derivatives from polysaccharides and extractives of the wood species used. The inorganic component is composed of chemical pulping chemicals and their transformation products as well as the ash components of the wood species. Direct application of spent liquors to the soil can have severe consequences on the biological activities of soil micro organisms as well as plant functions. It, therefore, is quite important to isolate and purify the lignin from the spent liquor. In alkaline pulping liquor, the lignin is precipitated by lowering the pH. The isolation of lignosulphonates directly from the spent sulphite liquor requires much effort and results in increased costs.

iii. Technical lignins and those in the form of spent liquors contain insufficient amounts of macronutrients, especially nitrogen and phosphorus.

The last point is even more important, especially for the proper functions of micro organism in the soil and the development of plants. Technical lignins are devoid of nitrogen and hence can only be used as artificial humus if nitrogen is added. The C/N ratio in lignin can be high as 100, which means that upon its application in the soil the nitrogen is completely utilized by micro-organisms and hence not available to be used by plant. In general C/N ratios of more than 20 means micro-organisms use this nitrogen for the formation of biomass. Organic matter with a C/N ratio of lower than 20 has a lot to offer in terms of soil conditioning as well as supporting microorganisms in the soil. It is for these reasons that nitrogen has to be added into lignin before it can be used as a source of soil organic matter. This can be done in the laboratory in almost the same way as it happens in nature but faster, resulting in a product with similar properties as natural humus. However, before explaining how this can be done it is important to discuss briefly how this process takes place in nature and point out similarities and differences between the two processes. Before describing the role of lignin in detail, it is important to describe the material itself and the various processes used to isolate. This will serve as the background for understanding the various lignins that can be used for the manufacture of humic materials as well as their behaviour.

2.6.1 Lignin: description

Lignin is the second most abundant organic compounds on earth after cellulose. It is an integral part of the cell walls of plants, especially in tracheids, xylem fibres and sclereids. In wood, it carries the major part of the methoxyl content, is unhydrolysable by acids, readily oxidisable and is soluble in hot alkali and bisulphite (Schubert 1965). It has several unusual properties for being a biopolymer, having a network structure and lacking a defined primary structure (Anonymous 1987). There are two ways to describe lignin: 1) from a chemical point of view i.e. its functional group and lignin-type sub-structure compositions and 2.) from a functional point of view that stresses what lignin does within a plant (Hatfield and Vermerris 2001).

2.6.1.1 Structure and biosynthesis

Lignin is formed through a process called lignification, a process by which various types of substructures called phenylpropanoid compounds (p-hydroxycinnamyl alcohols) are randomly polymerized to form a complex structure joined together by C-O-C (ether) and C-C linkages (Fengel and Wegner 1984). There are three main types of p-hydroxycinnamyl alcohols i.e. para-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (figure 2-3). The biosynthesis of lignin starts with glucose derived from photosynthesis. It is converted to shikimic acid, the most important intermediate of the so-called shimic acid pathway. From this process, two aromatic amino acids are formed i.e. L-phenylalanine and L-tyrosine which are the starting materials for the enzymatic phenylpropanoid metabolism leading to the formation of the three cinnamyl alcohols (figure 2-3).

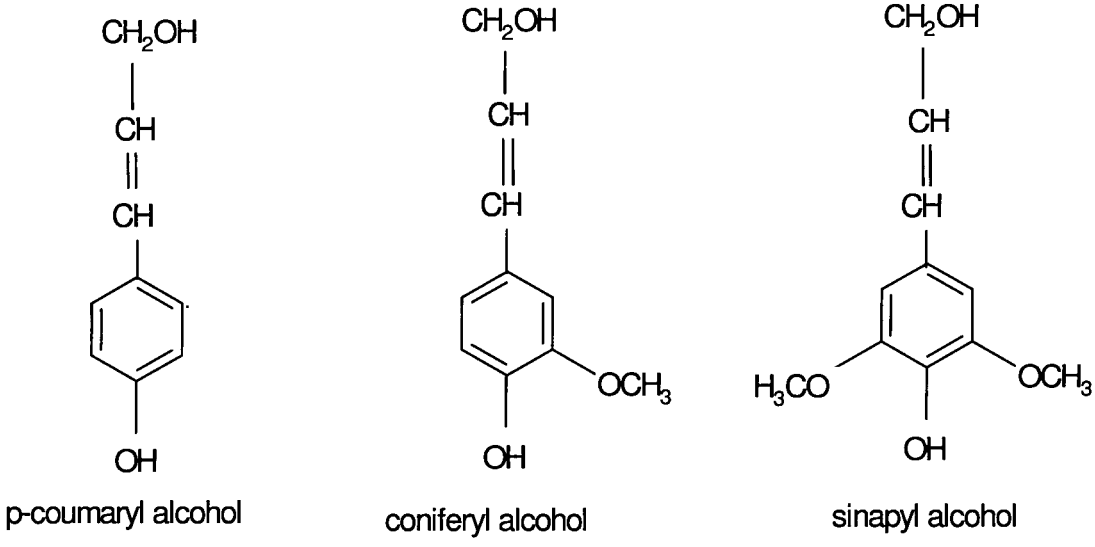

Figure 2-3: The building units of a lignin polymer.

The acids are deaminated by deaminases to their corresponding cinamic acids. The dominating further steps are successive hydroxylation and methylation leading to the formation of p-coumaric acid, which undergoes several transformation reactions before eventually forming sinapic acid. The cinnamyl alcohols are eventually formed by

enzymatic activation and reduction of the corresponding acids via co-enzyme-A thioesters and aldehydes. The biosynthesis of lignin macromolecules from the monomeric units takes place through dehydrogenative polymerisation of p-hydroxycinnamyl alcohols to form dimeric structures of which some of the most prominent are shown in figure 2-4 below.

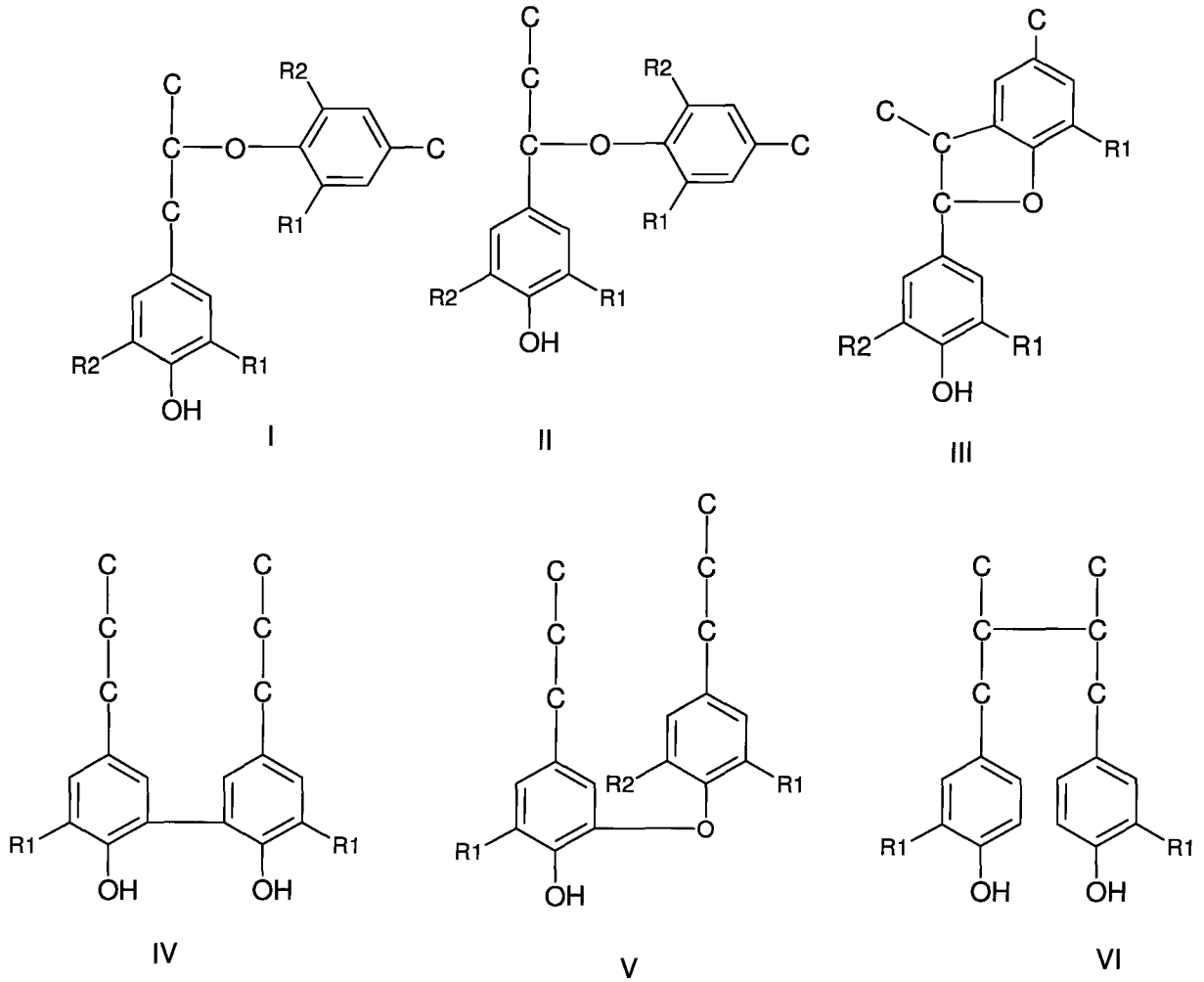

Figure 2-4: The dominating lignin bond types: β-O-4' (I), α-O-4' (II), β-5' (III) 5-5' (IV), 4-O-5' (V), and β-β' (VI).

The further polymerisation is called end-wise polymerisation involving coupling of monolignols (see figure 2-5) with phenolic end groups of di- or oligolignol or a coupling of two end-group radicals, yielding a branched polymer via tri-, tetra-, penta-

and olignols. Figure 2-6 presents a hypothetical structural formula of a softwood lignin segments proposed by Adler (1977).

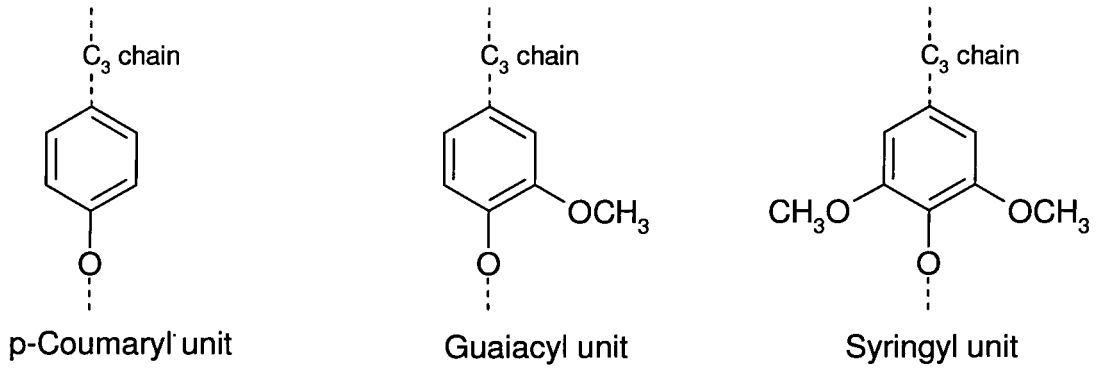

Figure 2-5: Monolignol units of lignin.

Gymnosperms as in Norway spruce have a lignin that consist almost entirely coniferyl alcohol. Dicotyledonic lignin is a mixture of coniferyl and sinapyl units and monocotyledonic lignin contains all of the three monolignol derived units. Some monocotyledons have mostly coniferyl units (as many grasses; grasses have also higher amounts of p-coumaryl substructures) while other have mainly sinapyl units, as some palms.

In a wood cell, in the middle lamella the lignin content is high but because this layer is relatively thin (0.1-1.0µm in thickness); only 20%-25% of the total lignin in wood is found here. Most of the lignin is found in the secondary wall of the cell due to the large volume of this layer compared to other layers. The primary wall is a thin layer, 0.1-0.2µm, and is also composed of lignin embedded between cellulose, hemicelluloses, protein and pectic substances (Fengel and Wegner 1984).

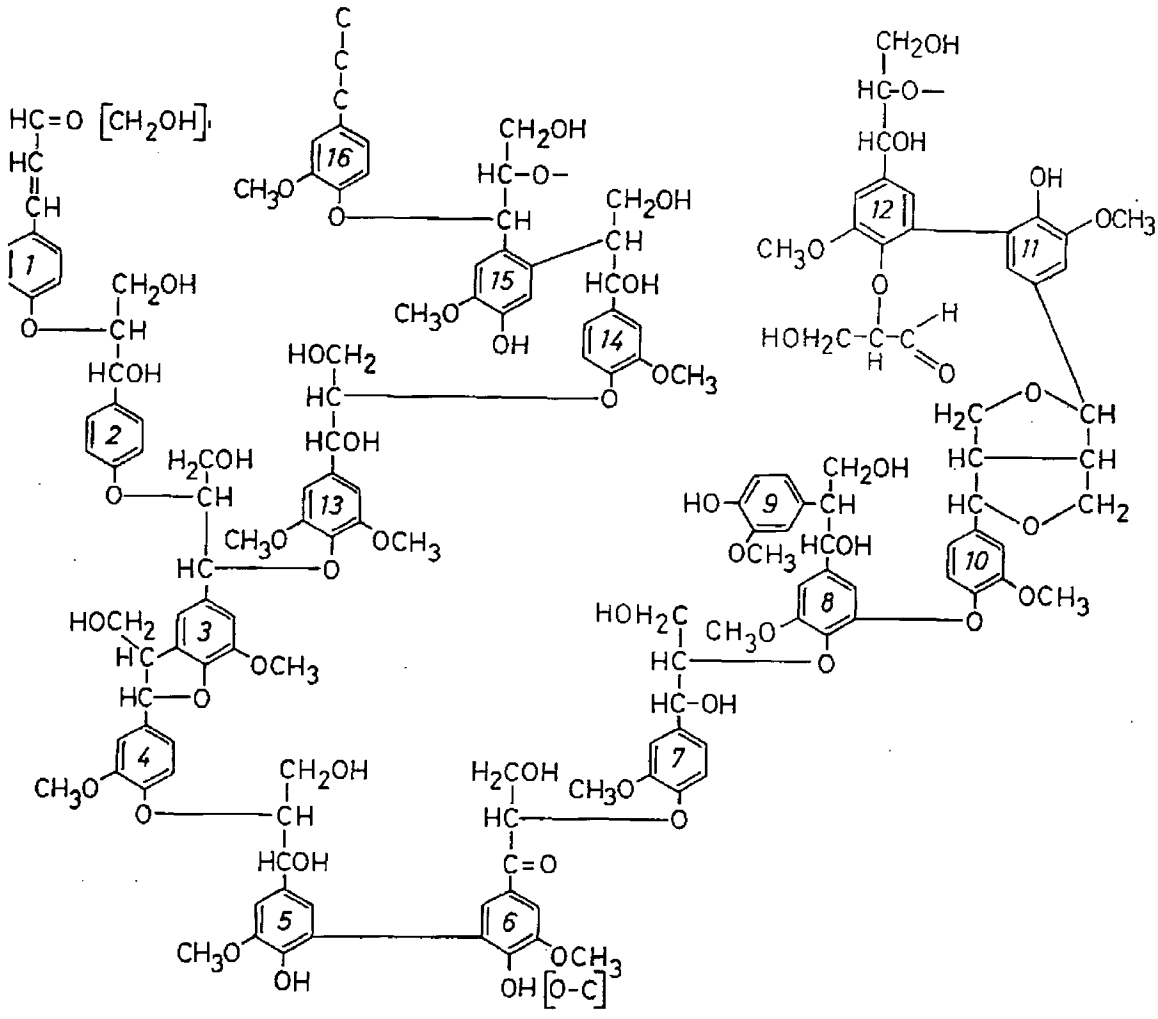

Figure 2-6: A structural segment of a softwood lignin as proposed by Adler (1977).

2.6.1.2 Lignin functions

Lignin is a natural bonding agent when combined with hemicellulose, for the cellulose fibres in wood. It imparts rigidity to cell walls and in woody parts acts as a permanent bonding agent between cells, generating a composite structure outstandingly resistant towards impact, compression and bending. Indeed, it is in this way that lignin provides strength and rigidity to tissues of vascular plants especially to woody plants. Lignin also protects the polysaccharides from microbial attack by impeding penetration of destructive enzymes into cell walls, and is involved in water transport, nutrients and

metabolites by decreasing the permeation of water across cell walls in the conducting xylem tissue (Freudenberg 1968). The polysaccharide components of plant cell walls are highly hydrophilic whereas lignin is more hydrophobic. The crosslinking of polysaccharides by lignin is an impediment for water absorption to the cell wall. Thus, lignin makes it possible for plants to form vascular tissues which conduct water efficiently.

Lignin, due to its structure and high molecular weight – serves as UV protection and flame retardant agent. The latter property is of benefit in forest fires when the special lignin-carbohydrate complex is able to protect wood from the effects of fire (Wünning 2001).

It follows; therefore, that lignin may be used beneficially for many applications some of which are listed in the following subsection, but our discussion below first focuses on the isolation methods of different lignin preparations.

2.6.1.3 Lignin preparations

Approximately 40-50 million tons of technical lignins are produced worldwide (excluding Eastern European countries) as a by-product of wood pulping processes during the production of cellulose. About 95% of the lignin produced is burned to generate energy in pulp production plants. The remaining 5% is sold as lignosulphonate or Kraft lignin for various applications (Wünning 2001).

As there are various processes which are used to remove and isolate lignin from cell membranes of woody plants, the term lignin only defines the polymeric material located in these cell membranes. This is due to the fact that each process produces material of different composition and properties and because of these differences unique names are given to each lignin preparation and are applied when describing these materials. In the following section a glossary of various lignin preparations is provided. It is divided into two parts i.e. **pure analytical lignin materials** and **highly modified lignin materials produced using various industrial procedures**. The procedures used to isolate these

lignins result in the modification of the original structures and hence limit these lignin modifications to structural studies.

2.6.1.3.1 Analytical lignin preparations

Braun's lignin *(often referred to in early publications as native lignin):* is obtained by extraction of wood meal with 95% ethyl alcohol. Lignin yield in this procedure is very low (2-3%).

Cellulolytic enzyme lignin: This lignin preparation is isolated through the decay of finely ground wood meal with brown rot fungal species such as *Lentinus lepideus, Poria vailabtii* and *Lenzites sepiaria* followed by extraction with ethyl alcohol. Although this is a tedious procedure, relatively high yields of lignin are obtained (25-50%).

Dioxane acidolysis lignin: This type of lignin is isolated by the treatment of woody material with dioxane/dilute HCl. Certain linkages are cleaved by this procedure thus altering the lignin structure.

Milled wood lignin: It is isolated by stirring milled wood for two hours in a dioxane-water mixture. After removal of the solvent from the filtrate, the crude preparation is purified by dissolving into 1,2 dichloromethane-ethanol mixture (2:1 by volume) and precipitation into ethyl ether. The purified milled wood lignin requires extensive drying to bring it to water free condition. Yield is ~ 25% of the total lignin. Of the lignin preparations available, this is thought to best represent lignin found in wood and hence it has been widely used for studies on the chemical structure of lignin (Furuno et al. 2006).

Klason lignin: This lignin preparation is isolated through the treatment of milled wood with 72% H_2SO_4 at ambient temperature after the extraction of lipophilic compounds with organic solvents such as hexane, ethanol or acetone. After 2 hours reaction time,

two volumes of water are added; and the precipitated lignin is refluxed for 4 hours in 3% sulphuric acid to remove sulphuric acid ester groups and co-precipitated dextrans. This procedure is used to quantify the amount of lignin in woody materials. The lignin structure is markedly changed by the drastic conditions used in this treatment.

Periodate lignin: This lignin preparation is isolated through successive treatments of woody material with sodium periodate followed by boiling water. This removes carbohydrates from lignin. Lignin becomes partially oxidised, notably the units with free phenolic hydroxyl groups. Periodate converts such units into o-quinones, then to muconic acid structures.

2.6.1.3.2 Industrial lignin preparations

These lignins are co-products from processes designed to break down woody materials into fibrous materials and/or chemicals. These materials are often burned to produce energy or disposed through biological treatment systems. Some of these products are marketed commercially. All of these lignin products contain various amounts of non-lignin chemicals and most have been purified to some extent.

Hydrolysis lignin (Acid): This is a water-insoluble product produced by the strong acid hydrolysis of woody material to produce sugars for fermentation to alcohol. The resulting lignin is highly altered structurally and contains a large amount of sugar degradation products, wood extractives, and inorganic compounds. Much effort has been applied in developing useful products from this material particularly in the countries of the former USSR.

Kraft lignin: The vast majority of chemical pulp produced worldwide is generated through the Kraft pulping process. Lignin is removed from woody material in a reaction with NaOH and Na_2S at temperatures of 155-175°C. Although almost all of the lignin generated from this process is burned to produce electricity and steam, and to regenerate cooking chemicals, a small amount is isolated through acidification or ultra filtration and sold. This water-insoluble product is almost entirely lignin although

highly modified. A large percentage of the Kraft lignin market is a water-soluble sulfomethylated form.

Lignosulphonates (from acid sulphite pulping): Woody material is reacted with sulphur dioxide and a metal bisulphite at pH 1-2 and a temperature between 125-145°C. The water-soluble lignosulphonates produced contain sulphonated lignin polymers, sugars, sugar acids and small amounts of wood extractives and inorganic compounds. Approximately a million tons of lignosulphonates made by this process are sold each year, making it easily the largest source of commercial lignin products.

Lignosulphonates are produced as sodium, calcium, magnesium or ammonium salts and can be converted to other salts through base-exchange procedures. Changing the base of a lignosulphonate product will often affect how it performs and have significant effect on its physical properties. The two most common forms, sodium and calcium salts, have many different governmental approvals including use in food and feed, as anti-scaling agents, boiler water additives and binding agents for animal feeds.

The properties of lignosulphonates are also affected by whether they are produced from softwoods, hardwoods or grasses. Hardwood and grass lignosulphonates for example generally have lower molecular weight than softwood lignosulphonates.

Lignosulphonate (from bisulphite pulping): Woody material is cooked at a pH of 3-5 at 150-175°C with a metal bisulphite salt. The water-soluble material produced contains 40-50% sulphonated lignin. The remaining material consists of sugar polymers, sugars, sugar acids, wood extractives and a large amount of inorganic compounds. Lignosulphonates from this process are currently not sold commercially.

Lignosulphonates (from the neutral sulphite semi - chemical process): Woody material is treated with salts of bisulphite/sulphite at pH 6-9 prior to mechanical refining. The water-soluble material that is produced (in very low yields) contains less than 50% sulphonated lignin and a variety of sugar degradation products, wood

extractives and a large amount of inorganic compounds. A small amount of this material is sold commercially.

Lignosulphonates from alkaline sulphite - anthraquinone pulping: Woody material is reacted with sodium sulphite and a catalytic amount of anthraquinone at pH 9-13 and 160-180° C. The resulting water-soluble lignosulphonates contain less than 50% sulphonated lignin, a variable amount of wood extractives and a large amount of both sugar acids and inorganic compounds. These are not currently commercial products.

Organosolv Lignin: Lignin products produced from a number of different organic solvent-based systems are referred to as organosolv lignins. The water insoluble products produced in these systems are usually purified and contain a very high percentage of lignin. Many different modified lignin products have been developed from organosolv lignins including water-soluble sulphonated materials. These lignin products are currently not marketed because of unfavourable economics associated with the production of pulp using these systems. The following are the four major organosolv processes:

Alcell: Ethanol/water pulping.
ASAM: Alkaline sulphite anthraquinone methanol pulping.
Organocell: Methanol pulping followed by methanol, NaOH and anthraquinone pulping.
Acetosolv: Acetic acid/HCl pulping.

Steam explosion lignin: Woody material is separated into fibres through high temperature/high pressure treatment with steam. The lignin material that is obtained contains low level of carbohydrate and wood extractive impurities. The water-insoluble lignin itself has a somewhat reduced molecular weight caused by acid hydrolysis reactions. This type of system is often used along with enzymatic hydrolysis to produce sugars for fermentation.

2.6.1.4 Applications and use of lignin as a raw material

Lignosulphonates

Cleansed and refined lignosulphonates are used for the following applications:

- As an additive in concrete admixtures. Lignosulphonate is added to concrete as a water reducing and/or air-entrapping additive; this increases the compressive strength and frost resistance as well as increasing the concrete flow properties. The addition of lignosulphonate also has a dispersing effect on the suspended cement particles.
- As an additive to animal feed. Lignosulphonate is added as a bonding agent, thus improving the pellet quality and production efficiency. Due to the excellent bonding qualities of the lignosulphonate, animal feed pellets become harder and dust development is reduced.
- As an additive to duroplast. Lignosulphonate is added to aminoplast or phenoplast glues. Lignosulphonate improves the adhesive properties at low temperature.

Lignosulphonates have a number of other uses, due to mainly their properties (dispersing, bonding/gluing).

These include:

- additives for the production of composite colours
- as additives in crude oil well drilling muds where they function as conditioners and provide the required viscosity, yield point, gel strength, shale stabilisation and fluid loss control at high temperatures.
- as a chemical raw material for the synthesis of vanillin.
- for the production of briquettes from coal products.
- as an expander in lead-acid batteries.
- as an additive in rubber products.

Alkaline lignins (Kraft lignins)

In the past, no interest was shown in the distribution and use of Kraft lignins to produce other goods and the liquors were combusted by choice. Consequently, much less

research has been carried out on Kraft lignins than on lignosulphonates. The few known applications of Kraft lignins were related directly to their pronounced phenolic character, their main use being as a condensation partner in phenol formaldehyde resins.

2.6.2 The role of lignin in the formation of soil organic matter (SOM)

2.6.2.1 SOM: Description

The term "soil organic matter" refers to the sum of all organic carbon containing substances in the soil. SOM consist of a mixture of plant and animal residues in various stages of decomposition, substances synthesized microbiologically and /or chemically from breakdown products, and the bodies of live and dead microorganisms and their decomposition remains (Schnitzer and Khan 1978). There seems to be some confusion among soil chemists about the meaning of SOM, humus and humic substances. According to Schnitzer (2000), the term total humic substances is synonymous with SOM and humus. In this investigation humic substances is defined as the sum of humic acids + fulvic acids + humins. Humic substances make up a large portion of the dark matter in humus. Humic substances are chemically complex, colloidal, supramolecular acidic mixtures, predominantly aromatic, hydrophilic, polyelectrolyte-like materials that range in molecular mass from a few hundreds to several thousands.

As a lot of different humic molecules, in very diverse physical associations, are mixed together in natural environments, it is difficult to measure their exact concentrations and allocate them to a certain class of bio-organic molecules. Humic acids are usually classified into the following three main fractions according to their pH dependent solubility:

i. Humic acid (HA), which is soluble in dilute alkali but is precipitated on acidification of the alkaline extract.
ii. Fulvic acid (FA) which is that humic fraction which remains in solution when the alkaline extract is acidified i.e. it is soluble in both dilute alkali and acid;
iii. Humin, which is that fraction that cannot be extracted from soil or sediment by dilute base or acid.

These three fractions may distinctly differ in molecular mass and functional group content with FAs usually having the lowest molecular weights, containing more oxygen but less carbon, and having a higher content of oxygen containing functional groups (CO_2H, OH and C=O) per unit weight than the other two fractions.

2.6.2.2 Functions in soil and other uses

i. Humic substances exert physical, chemical and biological effects on soil quality by serving as soil conditioners, nutrient sources and substrates for microorganisms.

ii. They contribute to the maintenance of an adequate and stable soil structure by acting as binding agents in the formation of soil aggregates thus ensuring satisfactory drainage and aeration and in providing protection against erosion, enhancing the mechanical properties of the soil and playing a major role in water retention.

iii. They act as sources and store houses of N, P, and S and of micro-nutrients essential for plant growth. They form complexes with many metals and make these available to plant roots and microorganisms. They buffer soil against drastic changes in pH and also interact with herbicides and pesticides and assist in their degradation and detoxification.

iv. They serve as substrates for macro- and microorganisms in the soil. Soil microorganisms play an important role in the synthesis and degradation of humic substances. Humic substances can exert direct physiological effects on plants.

v. All' these affect the impact on soil quality and point to a vital role of humic substances in soil fertility (Schnitzer 2000).

Humic substances also find applications in medicine (Klöcking and Helbig 2001) as anti-microbial, anti-viral, anti-inflammatory and anti-tumor agents, liver stimulants, heal gastric ulcers, stop bleeding, treatment of skin burns, estrogenic agents and reduce heavy metal toxicity. In industry they find use as dispersants, corrosion inhibitors,

wood preservatives, etc. In agriculture they are used as fertiliser and spray additives, coating of seeds, nutrients in hydroponics.

2.6.2.3 Lignin humification

Humic substances arise from the chemical and biological degradation of plant and animal residues by the synthetic activities of microorganisms. This takes place mainly by reaction of mineral acids with carbohydrates to form furfurals, condensation of amino acids and peptides with carbohydrates to form melanoids, aldol condensation of amino acids with methylglyoxal, oxidation of phenolic substructures to quinone derivatives, and cleavage of aromatic lignin moieties (Haider et al. 1980). The products formed through these activities tend to be more stable than the corresponding starting materials.

As a source of phenolic compounds, lignin plays a key role in the natural humification process. There are various theories which have been developed to describe exactly how this occurs in nature (Schnitzer and Khan 1978) of which two of the most plausible are described below, i.e.:

i. The lignin protein theory: which states that lignin is incompletely utilized by soil microorganisms and can undergo a preliminary series of modifications including loss of methoxyl groups (OCH_3), generation of *o*-hydroxyphenols and termination of side chains to from carboxyl groups (COOH). The *o*-hydroxyphenols would further oxidize to quinones which are capable of undergoing condensation reactions with ammonia (NH_3) produced by the degradation of proteins by microorganisms and other N-containing substances in the soil. These would then first form humin, then HA and finally FA.

ii. The polyphenol theory: this most widely accepted theory is summarized in figure 2-7 below. It states that humic acids originate from lignin polyphenols. Polyphenols are then converted to quinones by polyphenoloxidase enzymes. Quinones then react with N-containing compounds and polymerise to form

humic macromolecules of higher complexity. The order of humic substance formation would then be FAs, then HAs and finally humins.

The formation of humic substances in soils, composts, peat bogs and water basins takes place both under aerobic and anaerobic conditions and is mainly driven by microbial processes. There are two main processes of microbiologically mediated humus formation i.e.:

i. The extracellular transformation of plants and animal constituents into humic compounds, a process which primarily involves lignin humification.

ii. The synthesis of humic precursors within the cells starting with simple aliphatic compounds. This synthesis is linked to the fundamental metabolism of microbes and indicates that humus may be formed as a metabolic by-product of carbohydrate metabolism (Fischer and Schiene 2002).

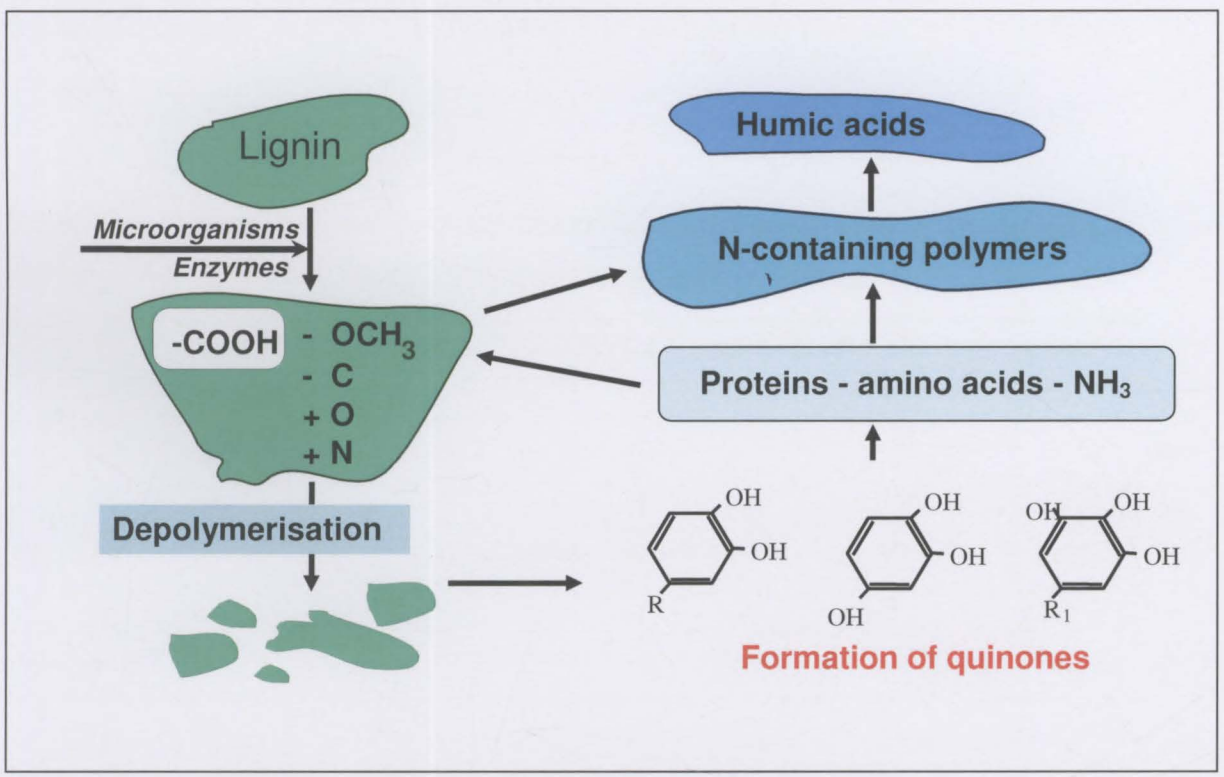

Figure 2-7: Schematic overview of the natural humification process (Fischer et al. 2002).

2.6.2.4 Chemical composition, properties and structure

Excellent reviews on the methods used to reveal the structure of humic acids have been given in numerous publications (Amblés 2001). The discussion on these methods will not be repeated here but a review of the main constituents as revealed by the various methods, the properties and structure will be reviewed.

Elemental compositions

Elemental analysis is probably the most commonly used method in the characterization of humic acids and provides information on the distribution of major elements. Humic substances contain carbon and oxygen as major elements in addition to hydrogen, nitrogen, sulphur and phosphorus. The range of elemental composition is relatively narrow. The carbon content varies from approximately 40 to 60% and that of oxygen from 30 to 50%. Nitrogen, sulphur and phosphorous contents are much lower varying from 1 to 4%, 0 to 2% and 0 to 0.003 % respectively (Amblés 2001). These values are further summarised in table 1 for HAs and FAs isolated from soils of widely differing climates.

Table 2-1: Average elemental compositions of HAs and FAs extracted from soils of widely differing climates adapted from Senesi and Loffredo (2001).

Element	Average (%)
Humic acids	
C	56.2
H	4.7
N	3.2
S	0.8
O	35.5
Fulvic acids	
O	44.8
C	45.7
H	5.4
N	2.1
S	1.9

Based on these data and disregarding S, average minimal formulas of $C_{10}H_{12}O_5N$ were calculated for HA and $C_{12}H_{12}O_5N$ for FA (Senesi and Loffredo 2001). Although these chemical formulas are not absolute values, they are useful to set boundaries for probable chemical structures to be confirmed by other chemical and physico-chemical properties. The elemental composition of HS is affected by factors such as pH, parent material, vegetation and age of the soil.

Functional groups

The main functional groups in FAs and HAs are carboxyl and phenolic OH groups. Alcoholic OH and carbonyl (quinonoid and ketonic C=O) groups are also well represented whereas methoxyl (OCH_3) groups are found in smaller amounts. The average functional group compositions of HAs and FAs isolated from soils are given in table 2-2 on an ash and moisture free basis.

Table 2-2: Average functional group compositions of HA and FA extracted from soils of widely differing climates (Senesi and Loffredo 2001).

Functional group	**Average (cmol kg^{-1})**	**Average (cmol kg^{-1})**
Humic acids		***Fulvic acids***
Total acidity	670	1030
COOH	360	820
Phenolic OH	390	300
Alcoholic OH	260	610
Quinnoid and ketonic C=O	290	270
OCH_3	60	80

Cation exchange capacity

Humic substances are variable-charge soil components with a low point-of-zero charge of about 3. Thus humic substances (HSs) are negatively charged at pH>3. Since COOH and phenolic OH groups can deprotonate at pH common in many soils, COO^- and O^- are major contributors to the negative charge of soils and account for the ability of HS to retain cations in non-leachable forms prevalently by coulombic and

electrostatic forces. For this reason, HSs are considered a major contributor to the cation exchange capacity (CEC) of most soils especially organic soils. The capacity of HS for binding exchangeable cations is not fixed but varies widely according their chemical nature and properties. In general, the CEC of HSs increases dramatically with increasing pH due to increasing deprotonation and ionization of functional groups at higher pH.

Structural components

Humic substances consist of a chemically heterogeneous mixture of compounds and thus they cannot be regarded as single chemical entities described by unique chemically defined molecular structures. Although it is virtually impossible to describe uniquely the molecular formula of HA, FA and other HS fractions, it is possible to depict the general structures of a typical molecule of HA, FA on the basis of available compositional, structural, functional and behavioural data. The model structures such constructed contain the basic structural moieties and types of functional groups that are common to all the single, indefinitely variable unknown HS molecules. The structure of HA is naturally oxidized, as shown by the asterisks in figure 2-8 below, giving it a negative charge. Positive ions, attracted to broken bonds at the site of the oxidation, create sites for micronutrients and microflora to attach (Anonymous 2000).

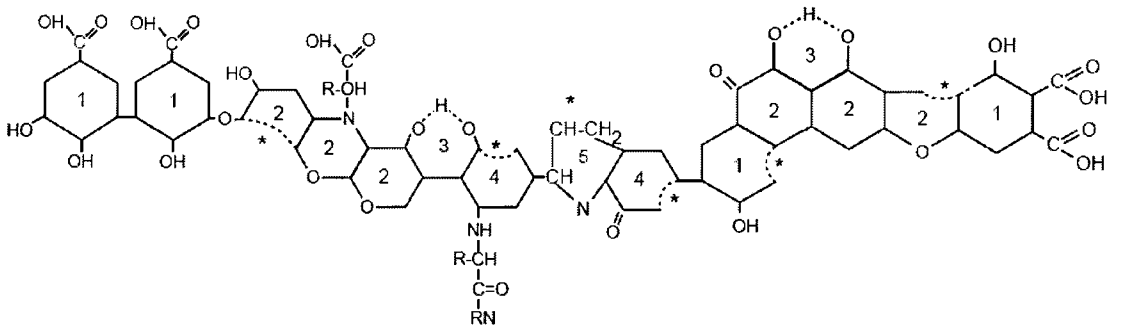

Figure 2-8: An oxidised humic acid molecule (Anonymous 2000).

The macromolecular structure consists of aromatic, phenolic, quinoide and heterocyclic building blocks that are randomly condensed or linked by aliphatic, O, N, and S bridges.

The macromolecule bears aliphatic, polysaccharidic, aminoacidic and lipidic chains as well as chemically active functional groups of various natures (mainly carboxylic, but also phenolic and alcoholic hydroxyls, carbonyls, etc.) which render the humic polymer acidic. The models feature a highly poly-electrolytic character and several sites and functional groups potentially able to bind metal ions, mineral surfaces and other organic compounds (Zhang et al. 1996).

2.6.3 The artificial humification of lignin by oxidative ammonolysis

The desire to employ lignin in the development and manufacture of humic materials is prompted by the following factors:

- There is a chemical similarity between lignin and humus as lignin (as discussed above) is one of the basic chemical components of humic matter in the soil.
- The availability of huge quantities of technical lignins (a by-product of wood utilization) mainly from the pulping industry. As the global pulp production has increased over the years so has the amount of technical lignins. These by-products are used mainly in energy production (Fischer and Schiene 2002).

Lignin has very little nitrogen to be considered as source of plant nutrients. Therefore, in order for lignin to be used for this purpose, nitrogen has to be incorporated. This can be done by reacting the lignin or lignin containing substance with ammonia, which acts as a source of nitrogen in the presence of an oxidative species, which can be air or oxygen. The process is termed ***oxidative ammonolysis, ammoxidation*** or simply ***N-modification of lignin.***

The products obtained through this process are called N-lignins. The major advantage of N-lignins is that the nitrogen is differentially bound in the lignin polymer and can be released periodically to plants. The types of nitrogen forms in order of decreasing degree of hydrolysability were classified by Wang et al. (1964) as mineral nitrogen, amide and strongly bonded nitrogen.

The oxidative ammonolysis of technical lignins proceeds in a similar manner as in the natural environment during the formation of HS. However, in this case the time is greatly shortened. This process is summarised in figure 2-9 below. As the figure illustrates, the reaction is characterised by demethoxylation and demethylation of the lignin, formation of quinones, oxidation of aliphatic side chains, formation of C-C bonds, aromatic ring cleavage and reaction and condensation with N (Fischer and Schiene 2002).

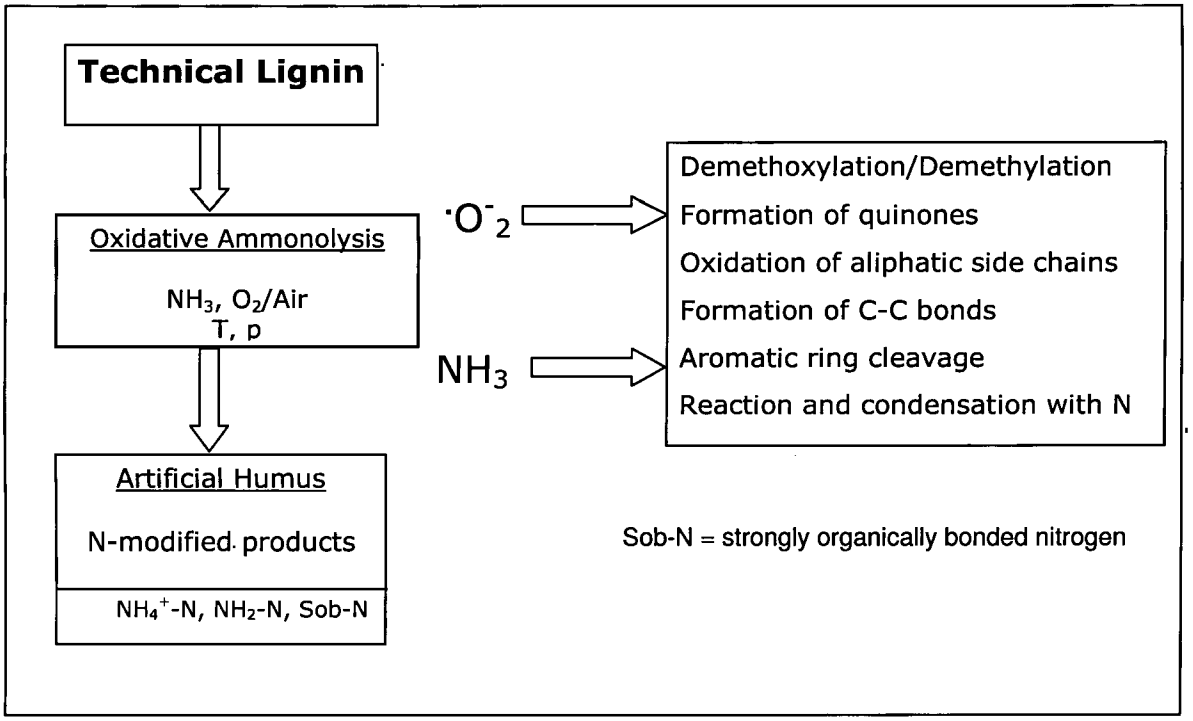

Figure 2-9: Overview of the artificial humification of lignin

It is important to describe how nitrogen is exactly incorporated in technical lignins. In general, the most important centres of reactivity in lignin are the α-C atom and the phenolic OH groups. In lignin there are two types of phenolic groups i.e. the etherified and the free phenolic groups. The degradation of etherified phenolic lignin structures takes place through the cleavage of β-aryl ether bonds as well as side chains. Ring structures containing nitriles and amides continue to exist. Aromatic rings are cleaved to yield dicarboxylic acids which are converted to ammonium salts and amides may be

formed. The release of CO_2 in the presence of ammonia leads to the formation of urea. The reaction starts with the nucleophilic attack of ammonia in the α-carbonyl groups of the lignin structures to give imines. Hydrogen is abstracted by hydroxyl radicals resulting in the cleavage of the α-, β-C-C bond formation of nitrile (applicable to the high pressure process). Nitrogen may be bonded to the aromatic rings via 1.2 addition of ammonia to the quinoide structures. The reaction scheme is illustrated in figure 2-10 below.

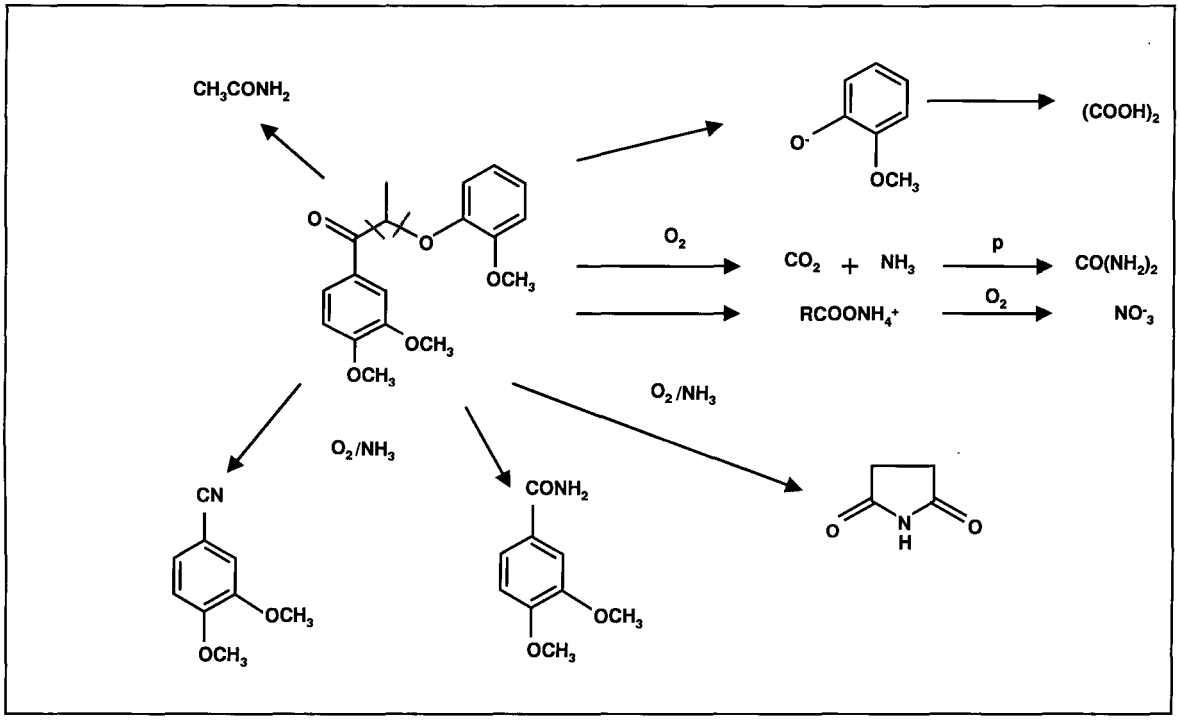

Figure 2-10: Possible pathway of the oxidative ammonolysis of lignin structures with etherified phenolic groups (Potthast et al. 1996).

In the free phenolic group containing structures, quinone methides can be formed in the ortho and para positions of quinone structures through alkaline demethylation/demethoxylation. As a result of formation of quinone methides, nucleophilic attack of the ammonia at the α-carbon is impossible because of the increased electron density at this point and hence the original phenyl propane structure remains intact. Through oxidative ring cleavage, dicarboxylic acids are formed from

which amides or imides can be formed. Ortho-quinones in the presence of ammonia react to form quinone imines which further react to form nitrogen containing polymers. New C-C bonds can develop and heterocyclic structures can be formed. The process is described in figure 2-11 below.

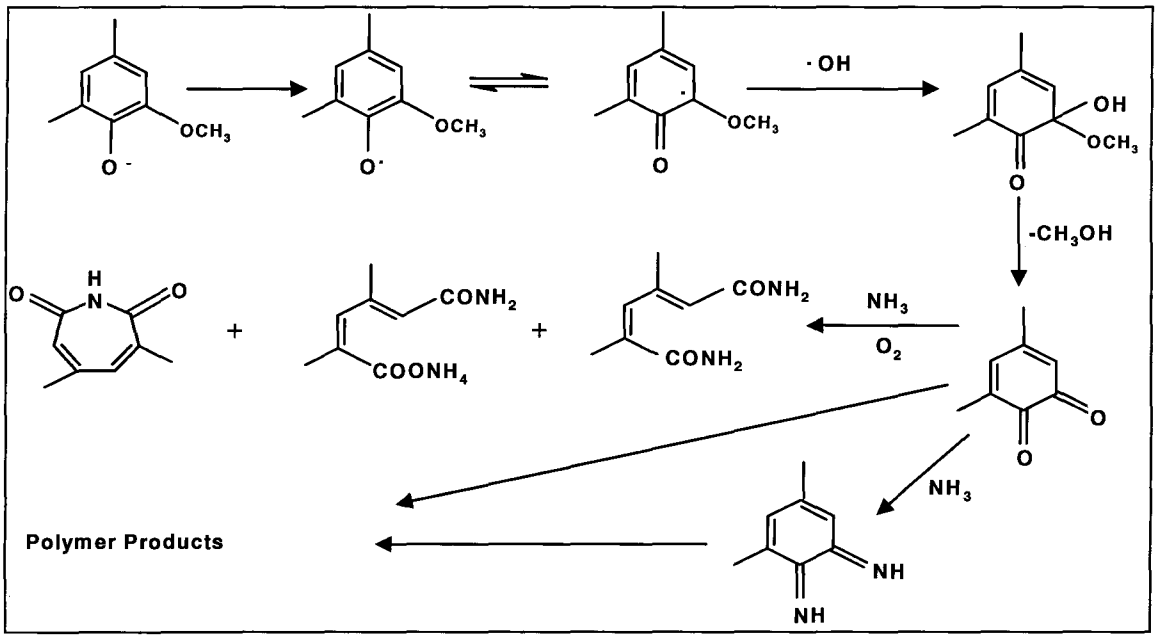

Figure 2-11: Possible pathway of the oxidative ammonolysis of lignin structures with free phenolic groups (Zakis and Neiberte 1978).

2.6.3.1 The development of a novel process for the manufacture of N-lignins

The oxidative ammonolysis of lignins was pioneered by Flaig (1960). A lot of effort had since gone into developing this process, employing technical lignins and lignin containing residues from various sources (Rozmarin and Valentin 1983; Fischer et al. 1990; Miletzky et al. 1990; Meier et al. 1992; Meier et al. 1994; Nascimento et al. 1994). A review of the historical development of the process was given by Meier et al. (1994), and more recently, Fischer and Schiene (2002) reviewed the chemical modification, properties and usage of N-modified lignins.

At the Institute of Plant and Wood Chemistry (IPC) of the Technical University of Dresden, Germany, initial investigations in this field focused on the use of technical lignins using air as an oxidant at high pressures in a bubble reactor with additional mechanical stirring, using spent sulphite liquors. The *N*-lignins produced contained up to 19% nitrogen. These studies greatly examined the influence of reaction parameters. The results indicated that the process is greatly controlled by temperature and oxygen supply. Within a range of 105 – 180 °C, N_{tot} increased proportionally with temperature from 11.5 - 18.5%. At the same time, the easily hydrolysable ammonium form of nitrogen decreased to about 30% while the organically bonded nitrogen increased to about 60%.

Investigations into kinetics of the process revealed that there is a direct relationship between the incorporation of nitrogen into the lignin macromolecules as well as the decrease in OCH_3 groups (Miletzky and Schiene 1985). The results pointed to a zero order reaction for both nitrogen incorporation and the decrease in OCH_3 groups with supply of oxygen to the reaction site, appearing to be the rate controlling step. Other studies on kinetics (Capanema et al. 2000; Capanema et al. 2001a; Capanema et al. 2001b; Capanema et al. 2002) revealed that the reaction temperature affects the reaction rate and that the nitrogen incorporation is linearly correlated with the oxygen uptake, CO_2 formation, oxygen incorporation into lignin, loss of carbon and methoxyl group content under all parameters such as oxygen pressure and during the entire reaction period. In addition to the process parameter discussed above, the ammonium concentration, the lignin type and base cation of the spent liquor have a significant effect on the total nitrogen that can be incorporated into the lignin.

It was however found that the properties of *N*-lignins are also affected by possible reactions with carbohydrates present in the reaction mixture. This was mainly due to the formation of heterocyclic products such as imidazoles and pyrazines, a fact also shown by Kort (1970) in the reaction of sugar with aqueous ammonia at increased temperature. In pot tests, *N*-lignins from conventional spent sulphite liquors prepared under high pressure, were found to contain physiologically active substances which had

negative effects on the growth and development of crops (Fiedler and Schmidt 1979; Fiedler and Schmidt 1981; Fiedler and Schmidt 1983).

In the next step of the investigations, a process was developed by which air or oxygen was fed into the reaction mixture under normal pressure at < 100 °C for up to 4 hours with up to 10% spent liquor and 1.4 – 8% ammonium water mixture. At this lower temperature, the amount of nitrogen incorporated was lower (up to ca. 11%). In addition, the ammonium nitrogen was higher and the strongly organically bonded nitrogen content lower. For this process, a good oxygen supply, temperature and a longer reaction time are determinants of the total nitrogen that can be incorporated in the lignin. In fact, in both the low and the high pressure processes, the total nitrogen and its distribution is dependent on temperature and time.

These studies formed the basis of the development NOVIHUM™; a patented novel artificial humus prepared with low grade coal. The motivation for using low grade coal was as a result of the similarities between brown coal and lignin, the fact that brown coal already contains more than 80% of HS, and the availability of huge quantities of this resource in Germany.

A small scale pilot plant located in Freienhufen ca. 100km from Tharandt was commissioned in January 2000 and after initial testing it has been used for the manufacture of NOVIHUM™ for pot and field trials as well as the optimization of the process for industrial scale production of the material.

NOVIHUM™ has successfully been tested in rehabilitation activities and initial greening of problematic sites in many areas around the world (Katzur et al. 2001; Katzur et al. 2002a; Katzur et al. 2002b; Katzur et al. 2002c; Katzur et al. 2002d; Katzur et al. 2003a; Katzur et al. 2003b; Liebner 2006). Studies by other workers have also shown that *N*-modified lignins have a positive effect on crops (Ramirez et al. 1997). These results from these studies have formed the basis for the development of an

industrial scale production plant of NOVIHUM™, which is due to start operating in the near future. The general properties of the material are listed in table 2-3 below.

Table 2-3: General properties of NOVIHUM™.

Shape	Granular (d_{90} 4mm)
Colour	Black
Compactness	$\approx 0.6 t/m^3$
Carbon	60-65 mass% (dry matter)
Nitrogen	5.5 mass% (dry matter)
Sulphur	0.6 – 0.7 mass% (dry matter)
Moisture content	About 20%
Ash content (%)	5-10 mass% (dry matter)
CECpot	30 – 60 mval/100g

2.6.3.2 Comparison between artificial and natural humus

The formation of artificial humus bears similarities with the natural humification process as far as macromolecular building principles of the aliphatically linked aromatic units, in the O-functional groups and the N-modification in the form of amide, nitrile and amine functional groups including heterocyclic structures, are concerned. However, the reaction time is shorter. Table 2-4 below shows both the similarities of the C and N contents (thus the C/N ratios) as well as the N–distribution (%of N_{tot}). The artificial humus (N-lignins) contains fewer COOH groups thus their CEC is lower than the natural humic substances.

Table 2-4: Comparison between the structural properties of artificial and natural humus (values given on a dry matter basis).

Humus Type	C%	N_{tot} %	NH_4^+-N* (%)	Amide-N* (%)	Sob N* (%)	OCH_3 (%)	CECpot mval/100g
Artificial	49 – 59	3 – 5	10 – 41	14 – 21	44 – 73	10 – 12	140 – 180
Natural	41 – 62	1 – 5	10 – 25	21 – 45	Ca. 50	0.9 – 1.8	180 – 500

* Calculated as a percentage of total nitrogen.

2.6.4 Industrial residues from S.A. as raw materials for N-lignins

Three million tons of crushed sugar cane bagasse residues from sugar mills are available in South Africa annually (Ysbrandy 1992). Some of this lignocellulose finds its way back into sugar mills as a cheap source of energy source along with coal. Two large industries which successfully use bagasse residues, are the pulp and paper industry for the production of pulp used in the manufacture of coated paper as well as the particle board industry which uses selected bagasse particles in the manufacture of interior grade particle boards (Ysbrandy 1992).

Bagasse is used locally by Illovo Sugar, a leading, global, low-cost sugar producer and a significant manufacturer of high-value downstream products such furfural*. In general, the by products of sugar mills are molasses which the chemical division of this company converts into fermentation products, and bagasse which is the raw material for the furfural plant. The furfural reaction is an autohydrolysis reaction whereby residual pentosans present in the bagasse are transformed into furfural. This reaction is driven by high pressure steam and catalyzed by carboxylic acids which are generated internally. During this reaction, the lignin becomes depolymerised and can be recovered from the partially stripped lignocellulosic residue by solvent extraction with weak alkali. After decanting, the solution is acidified to precipitate the lignin, which upon drying forms a light brown powder. The solubility characteristics of this lignin are typical of Kraft lignin but the methoxyl content is much lower. This supposedly should result in a large number of reactive sites and hence open up a number of possibilities for utilization of this lignin (Reimann 1990; Ysbrandy 1992).

A pilot plant was constructed to demonstrate the process and to provide samples for commercial application trials. This so called "autohydrolysis lignin" was trade named

* Furfural finds direct applications as a selective refining solvent for lubricating oils, vegetable oils and manufacture of resins (phenol furfural resins). About two-thirds of world production of furfural is converted into derivatives such as furfural alcohol and tetrahydrofuran. Possible alternative applications for furfural derivatives are antibacterials and pesticides, Sain, B. (1983). "Furfural and Furfural Based Chemicals." J. of Sci. and Ind. Res. **41**: 431 - 438.

Sucrolin and is commercially available on the speciality chemicals market and is marketed by the Aldrich Chemical Company (cat. No. 37, 107-6) (Lora and Glasser 2002).

Along with Sucrolin, other lignin-based materials such as lignosulphonates are produced locally through a joint - venture between Sappi (through their Saiccor plant), the world's largest producer of coated fine paper and Borregard of Norway, the world leader in lignin based products through its subsidiary, Borregard LignoTech. The main objective for establishing this joint venture was to generate revenue from a previously unused component of Sappi Saiccor's pulping process normally directed to effluent which was discharged into the sea through a 6km marine pipeline.

In December 1998, a production plant was commissioned in Umkomanzi, south of Durban, trading as LignoTech South Africa, under the auspices of this joint venture. LignoTech South Africa uses advanced lignin beneficiation technology which involves evaporation processes, chemical processing and spray drying to produce a range of speciality lignin chemicals with unique and important applications such as additives in concrete, brick and ceramics production, binding agents in animal feeds and as dust suppression agents. Through this joint venture not only a new revenue source was created but also environmental impact on the marine environment of Saiccor's effluent was reduced.

LignoTech South Africa expanded in 2003 from an initial annual capacity of 55,000 tonnes of lignin products, to 155 000 tons per annum at a cost of R330 million. This therefore makes LignoTech South Africa the world's largest single producer of speciality lignin chemicals, and earns more valuable foreign exchange for South Africa as well as create new jobs both directly and indirectly, of benefit to the local economy (TAPPSA 2005).

Preliminary studies by Tyhoda (2003) aimed at screening raw materials from South Africa including low grade coal have shown the potential of the use of these materials in the manufacture of N-lignins. However, the process used in those studies involved the use of pressurised oxygen which is in contrast with the currently used method which uses air or oxygen under ambient conditions. This process is however very useful as a screening method. The current study is aimed at the use of the ambient procedure in the development of N-lignins using the patented ambient procedure used in the manufacture of NOVIHUM™.

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3.0 Structural characterisation of South African technical lignins by Curie-point Pyrolysis GC/MS

Abstract

Curie-point Pyrolysis GC/MS was applied to study the characteristics of South African technical lignins derived from the sulphite pulping process (a calcium lignosulphonate), sugar cane bagasse (Sucrolin), and a partially stripped sugar cane bagasse (a lignocellulosic residue with a low lignin content). Pyrolysis GC/MS revealed that the bagasse lignocellulosic residue, as expected, had a high content of carbohydrate material (43.6%) and low lignin content (29.0%). In comparison, the calcium lignosulphonate also had a high content of carbohydrate compounds (36.8%) resulting from the hemicellulosic material dissolved in the liquor during pulping (pyrolytic lignin content 56.9%). The Sucrolin had the lowest amount of carbohydrates (1.8%) and the highest content of lignin (86.9%) which is characteristic of steam explosion lignins. As they had common origin, the lignocellulosic residue and Sucrolin had similar p-hydroxyphenyl- : guaiacyl- : syringyl unit ratios (H:G:S ratios) i.e. 36:53:11 and 36:50:14, respectively. The calcium lignosulphonate mainly consisted of guaiacyl units with a H:G:S ratio of 9:71:20, typical of *Gymnosperm* lignins. In comparison, Indulin AT™, a Kraft lignin which is a mixture of softwoods and hardwoods consisted almost exclusively of guaiacyl units (95%, G). Analytical pyrolysis proved that the Sucrolin is a lignin of high purity and its properties augurs well for many biological and chemical treatments or modifications, including oxidative ammonolysis.

3.1 Background

Most of the methods used in the isolation of cellulose from lignin during the pulping process seek to dissolve the lignin. As a result, lignin is always obtained in an altered form and hence technical lignins from different sources always have different properties and hence different reactivities during any biological or chemical modification or

treatment (Dence and Lin 1992). It is therefore imperative that lignins should be characterised prior to any such applications.

One of the most convenient and frequently used methods for characterising technical lignins and similar ligneous materials is Curie-point Pyrolysis GC/MS. This method employs standardized thermo-chemical degradation processes. The degradation processes result in the fragmentation of the polymers. The structure of the polymers can be deduced from the fragments so formed. Curie-point pyrolysis-GC/MS, therefore, belongs to deductive methods for structural analysis.

Combined with a gas chromatograph, the pyrolyser serves as a sampling system. Its function is to convert the sample into a complex mixture of gaseous products which exhibit the characteristics of the original polymer. Pyrolysis is achieved in the absence of oxygen and at a defined working temperature. Curie-Point Pyrolysis takes place under reproducible conditions, which are required for quantitative evaluation. A sample container made of a special alloy is heated within a few milliseconds to the Curie point temperature. This is the temperature where the alloy loses its magnetic properties. The inductive heating results from a high frequency voltage. The choice of the pyrolysis temperature has a great influence on the intensity of the pyrolytic degradation of the material. It is, therefore, mostly set to maximize the proportion of gaseous low molecular mass compounds and also to maintain the degradation as mild as possible so as to obtain maximum structural information. Another important factor influencing the results to a large extent is pyrolysis time. Longer pyrolysis times are necessary for complete separation of the constituent compounds of the analyte, especially in cases where the samples have low specific heat conductivity. However, shorter pyrolysis times are also necessary to avoid secondary reactions which complicates the interpretation of the chromatograms (Roschy and Liebner 2002; Tyhoda 2003).

After the pyrolysis and degradation of sample into its volatile degradation products, the products are separated by means of capillary gas chromatography. Structural

information on each of the individual compounds is obtained by mass spectroscopy after electron impact ionisation and fragmentation.

3.2 Materials and methods

3.2.1 Materials

- A bagasse lignocellulosic residue i.e. bagasse partially stripped from sugars for furfural production process (Illovo Sugar Ltd., South Africa).
- A bagasse lignin, trade named Sucrolin, which is produced through solvent extraction of bagasse with weak alkali (Illovo Sugar Ltd., South Africa).
- A calcium lignosulphonate sample (LignoTech, South Africa).

3.2.2 Method

Curie-point Pyrolysis GC/MS spectrometry

Curie-point Pyrolysis GC/MS was performed using a CPP-40 pyrolyser (Fischer/GSG) coupled with a GC 6890 and MSD 5973 (Agilent Technologies). 260 µg of the sample was pyrolysed at 600°C (FecralloyTM) for 10 seconds. The pyrolysate was carried by helium into the inlet (250°C, split 1:20) of the gas chromatograph. Separation was achieved using a fused silica Optima-5 column (30m, 0.25mm, 25µm), a column flow of 0.9ml/min, an oven programme starting with 50°C (5 min), then 5°C/min to 280°C (2 min), and an auxiliary temperature of 250°C. The mass spectrometer was operated in EI mode at 70 eV, 230°C and $1.5 \cdot 10^{-5}$ Torr.

3.3 Results and discussion

3.3.1 Pyrolysis products

Altogether 90% of the total peak areas obtained by TIC integration could unambiguously be assigned to specific organic compounds. 75 to 100 compounds were assigned in each raw material. Most of them were in turn grouped according to lignin origin as well as their lignin building units (p-hydroxyphenyl (P), guaiacyl (G) and syringyl (S) units) or carbohydrate fraction (table 3-1). However, when calculating their pyrolytic lignin portion it has to be taken into account that the same amount of any two

compounds in the pyrolysates may give different results in MS detection. To get correct values, the response factors of all individual compounds contained in pyrolysates have to be known. This is rather difficult with pyrolysis-GC/MS, since a couple of unknown products are usually formed under pyrolytic conditions. Hence, the calculated pyrolytic lignin and carbohydrate contents should be considered as approximate values having a possible error range of about 5% according to response measurements done on a number of low-molecular substances obtained during thermal lignin degradation (Scholz et al. in press).

At the beginning of the pyrolysis, the gas peak appeared after the dead-time (time from start to the appearance of the first eluating compound) up to a retention time of 2.96min. Since this peak consisted of several co-eluating compounds (CO_2, CO, H_2O, SO_2, CH_4, NH_3, HCOOH, HCHO, CH_3COOH, CH_3OH etc.), its broad signal could not be resolved and no compound assignments were made. The total area was handled as a "gas peak" referring to all those compounds which are easily degraded under the specific pyrolytic conditions.

Figure 3-1 presents a total ion chromatogram (TIC) of the Curie-point pyrolysis GC/MS data of the Sucrolin and the lignocellulosic residue. Pyrolysis GC/MS revealed that the lignocellulosic residue, as expected, had a high content of carbohydrate and a low content of lignin material (table 3-2). The cellulosic material can also be seen in the micrograph presented in figure 3-2 after the lignin was removed. As the figure 3-1and table 3-3 demonstrate, the lignocellulosic residue and the Sucrolin had similar H:G:S ratios (36:52:11) and (36:50:14) respectively due to the fact that they had a common origin. In comparison, the calcium lignosulphonate and the Indulin AT (95%, G) (Liebner et al. 2006b) mainly consisted of guaiacyl units. The calcium lignosulphonate had a H:G:S ratio of 9:71:20 (table 3-2).

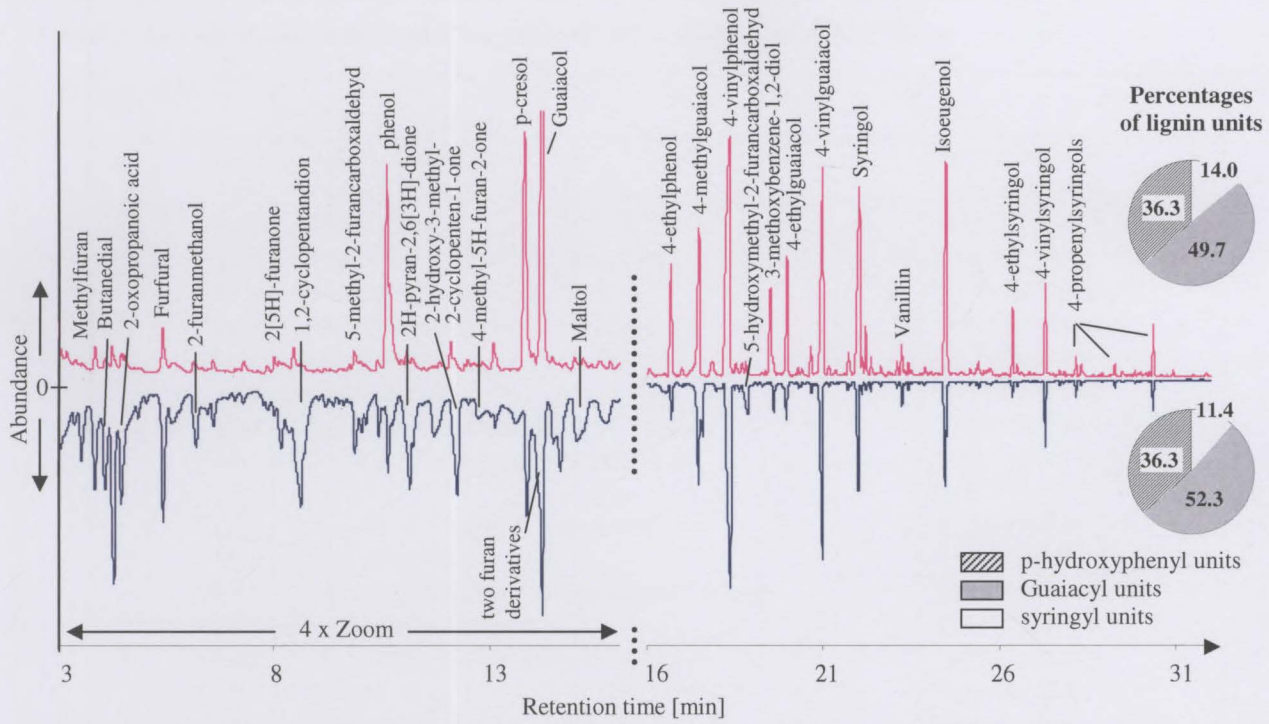

Figure 3-1: Total ion current chromatogram of the Curie-point pyrolysis GC/MS data of Sucrolin (positive abundancies) and lignocellulosic residue (negative abundancies). Peak origin of compounds in the peaks are listed in Table 3-1.

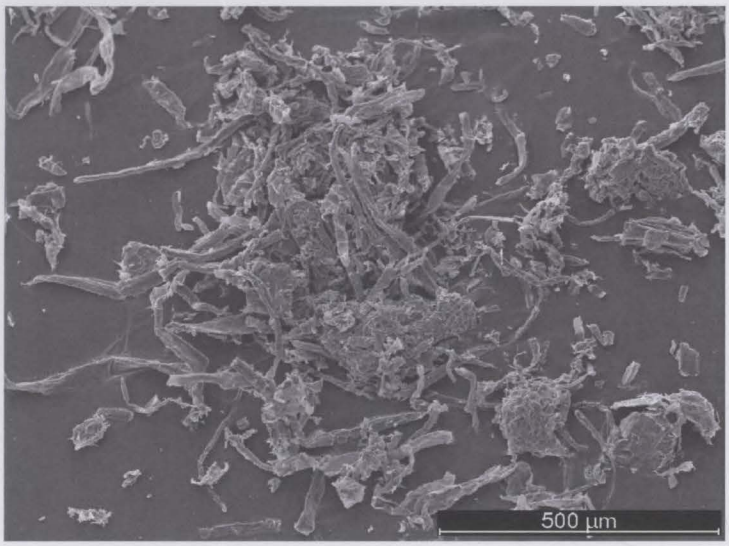

Figure 3-2: Micrograph showing the cellulosic material after the lignin was removed.

Table 3-1: Lignin derived products of Curie-point Pyrolysis at 600°C

Retention time [min]	Compound name	Origin
2.98	2,5-Dimethylfurane	carbohydrates
3.85	1-Hydroxy-2-butanon	carbohydrates
4.56	Acetylfurane	carbohydrates
5.43	Furfural	carbohydrates
6.14	Hydroxymethylfurane	carbohydrates
7.85	2-Methyl-2-cyclopenten-1-one	carbohydrates
8.02	Acetylfurane	carbohydrates
8.13	2(5H)Furanone	carbohydrates
8.46	1,2-Cyclopentanedione	carbohydrates
9.87	5-Methyl-2-furane carboxaldehyde	carbohydrates
10.68	Phenol	P lignin units
11.08	2,2-Diethyl-3-methyloxazolidine	carbohydrates
11.85	4-Methylanisol	P lignin units
12.11	2-Hydroxy-3-methyl-2-cyclopenten-1-one	carbohydrates
12.43	Acetoxymethylfurane	carbohydrates
12.49	Furane carboxaldehyde	carbohydrates
13.82	p-Kresol	P lignin units
14.23	Guaiacol	G lignin units
14.96	Ethylanisol	P/E lignin units
15.78	Ethylphenol	P lignin units
16.08	2,4-Dimethylphenol	
16.31	3-Methylbenzofuranone+3-Methoxybenzaldehyde	
16.69	4-Ethylphenol	P lignin units
17	6-Methyl-Guaiacol	G lignin units
17.24	3-Methyl-Guaiacol	G lignin units
17.48	4-Methyl-Guaiacol	G lignin units
17.86	1,2-Benzendiol	carbohydrates / lignin
18.36	4-Vinylphenol	P lignin units
18.53	P derivative	P lignin units
18.71	Ethylmethylphenol	
18.81	5-Hydroxymethyl-2-furanecarboxaldehyde	carbohydrates
18.88	4-Methylveratrol	G/E lignin units
19.27	4-Allylphenol	P lignin units
19.37	4-Propylphenol	P lignin units
19.52	3-Methoxy-1,2-benzendiol	carbohydrates
19.98	4-Ethylguaiacol	G lignin units
20.3	4-(Prop-2-en-1-yl)phenol	P lignin units
20.4	4-Methyl-1,2-benzendiol	
20.65	*cis*-4-(Prop-1en-1-yl)phenol	P lignin units
21	4-Vinylguaiacol	G lignin units
21.36	Dihydroxymethylacetophenon	
21.73	*trans*-4-(Prop-1en-1-yl)phenol	P lignin units
22.03	Syringol	S lignin units
22.12	4-(Prop-2-en-1-yl)guaiacol	G lignin units
22.37	4-Propylguaiacol	G lignin units
23.11	*cis*-4-(Prop-1-en-1-yl)guaiacol	G lignin units

Table 3-1: Lignin derived products of Curie-point Pyrolysis at 600°C (continued)

Retention time [min]	Compound name	Origin
23.23	Vanillin	G lignin units
23.33	5-Methyl-1,2,3-trimethoxybenzen	S/E lignin units
23.43	*trans*-4-(Prop-1-en-1-yl)guaiacol	G lignin units
23.5	Dimethylmethoxyphenol	-
24.45	4-Methylsyringol	S lignin units
25.17	4-(Prop-2-en-1-yl)veratrol	G/E lignin units
25.41	4-Acetoguaiacol	G lignin units
26.38	4-Ethylsyringol	S lignin units
26.51	Guaiacylacetone	G lignin units
27.32	4-Vinylsyringol	S lignin units
28.18	4-(Prop-2-en-1-yl)syringol	S lignin units
28.34	4-Propylsyringol	S lignin units
29.28	*cis*-4-(Prop-1-en-1-yl)syringol	S lignin units
30.37	*trans*-4-(Prop-1-en-1-yl)syringol	S lignin units

Table 3-2: Proportions of the lignin and carbohydrate constituents of the technical lignins.

Technical lignin	Lignin constituents (%)	Carbohydrate constituents (%)
Sucrolin	86.90	1.81
Calcium lignosulphonate	56.93	36.85
Lignocellulosic residue	28.97	43.61

Table 3-3: Proportions of the lignin sub-group constituent of the technical lignins.

Technical lignin	p-hydroxyphenyl Units (%)	Guaiacyl units (%)	Syringyl Units (%)
Sucrolin	36	50	14
Calcium lignosulphonate	9	71	20
Lignocellulosic residue	36	53	11

To be able to assign all the compounds originating from pyrolysis products, standard compounds were run under the same chromatographic conditions in order to determine their retention time as well as to record their mass spectra. In cases where the standard compounds were neither available commercially nor be easily synthesized, the corresponding lignin pyrolysis products were assigned based on their fractionation behaviour and mass spectra of homologous substances. For example, the occurrence of 4-ethylsyringol in the pyrogram was found by predicting the mass spectrum of this compound based from the well-known mass spectra of 4-ethylphenol, 4-ethylguaiacol and 4-ethylsyringol (figure 3-3).

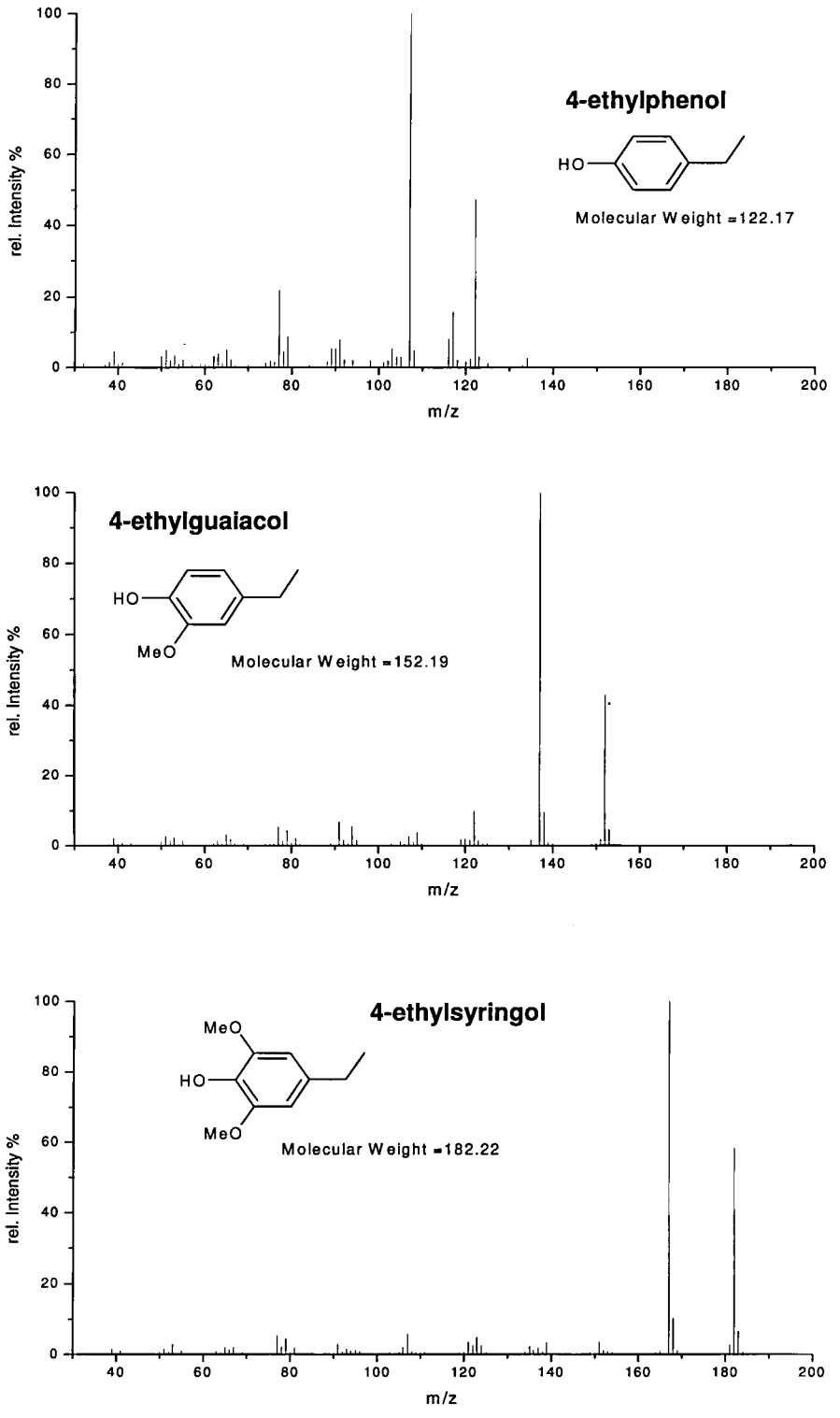

Figure 3-3: Mass spectra of 4-ethylphenol, 4-ethylguaiacol and 4-ethylsyringol.

The same procedure was used for determining the occurrence of the methyl alcohols 4-methylsyringol, 4-propylsyringol and 4-vinylsyringol (figure 3-4). It was also followed for 4-(prop-1-en-1-yl) phenol, 4-(prop-1-en-1-yl) guaiacol and 4-(prop-2-en-1-yl) syringol (figure 3-5).

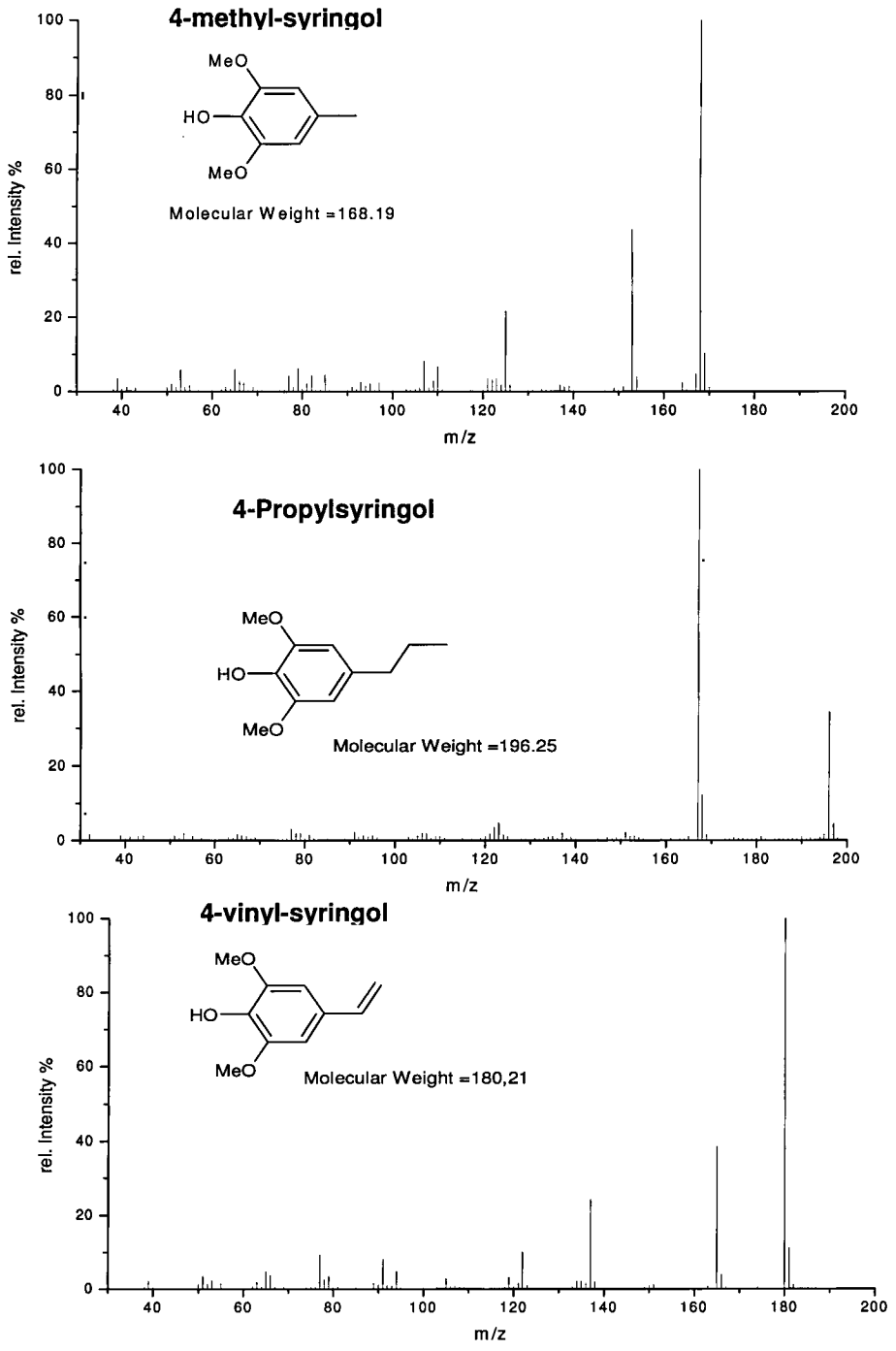

Figure 3-4: Mass spectra 4-methylsyringol, 4-propylsyringol and 4-vinylsyringol.

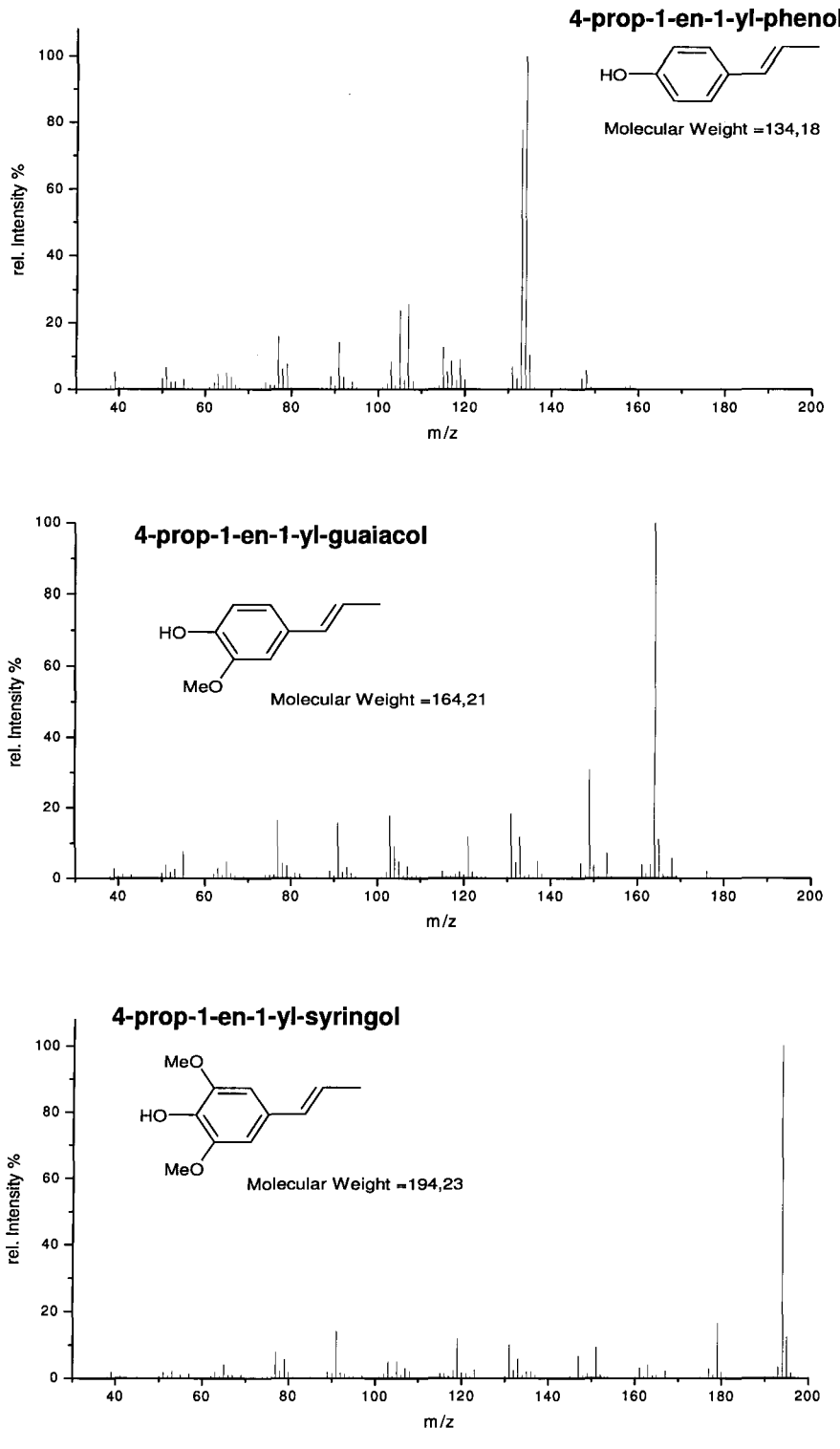

Figure 3-5: Mass spectra of allylphenols.

3.4 Conclusion

Curie-Point Pyrolysis GC/MS clearly revealed the structural differences between the three raw materials. The Sucrolin lignin, as also found in other studies, had a low content of carbohydrate components. Contrary to this, the calcium lignosulphonate had a high content of carbohydrate pyrolytic derivatives which is characteristic of the sulphite process from which it is obtained. There were similarities in the H:G:S ratios of the Sucrolin and the lignocellulosic residue due to common origin. However, the lignocellulosic residue had a higher content of carbohydrate compounds which were a result of the considerable high content of cellulose. Of the lignin components, the lignocellulosic residue and the Sucrolin had similar H:G:S ratios i.e. 36:52:11 and 36:50:14 respectively as they had common origin. On the contrary, the calcium lignosulphonate mainly consisted of guaiacyl units with a H:G:S ratio of 9:71:20, typical of *Gymnosperm* lignins. Curie-Point Pyrolysis GCMS is a powerful and rapid technique which does not require derevatisation of the compounds prior to analysis. It provides information on the amounts and compositions lignin and carbohydrate moieties of lignins and provides valuable information to explain or forecast lignin reactivity and other properties of industrial interest.

3.5 Literature cited

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4.0 Characterisation of the South African technical lignins by wet chemical methods

Abstract

Wet chemical methods were used to study the characteristics of South African technical lignins derived from the sulphite pulping process (a calcium lignosulphonate), sugar cane bagasse (Sucrolin), and a partially stripped sugar cane bagasse (a lignocellulosic residue with a low lignin content). Ultimate analysis revealed that the calcium lignosulphonate had a high ash (21.30%) and sulphur contents (4.93%) which were typical of the sulphite pulping process. It also had 10% reducing substances. These characteristics were typical of lignosulphonates as reported in literature. The high reducing sugar content, especially xylose, was a concern regarding the formation of product components which could render the nitrogen modified product toxic to crops. On the contrary, the Sucrolin had low ash and sulphur contents which were typical of technical lignins recovered from biomass conversion technologies. The Sucrolin was found to be highly oxidised which was a good material property as state of oxidation is central to the oxidative ammonolysis reaction. Therefore, higher N contents could be expected in products prepared from this material. The cellulose content of the bagasse lignocellulosic residue was not a concern as oxidative ammonolysis was not expected to solubilise into glucose.

4.1 Background

Most of the methods used in the separation of cellulose from lignin during the pulping process seek to dissolve the lignin. As a result, lignin is always obtained in an altered form and hence technical lignins from different sources and different pulping techniques always have different properties and hence different reactivities during any subsequent biological or chemical modification or treatment (Dence and Lin 1992). It is, therefore, imperative that technical lignins should be characterised prior to any such applications.

Characterisation starts with **ultimate analysis** i.e. the determination, by prescribed methods, of moisture and ash as well as the elemental composition of the organic portion of carbonaceous materials. Based on oven dry mass, the ash content of most woods is ca. 0.5% although it can be considerably higher for some species. The major constituents of ash are calcium, potassium, magnesium, sulphate, phosphate, carbonate and silicate with smaller amounts of other elements and ionic radicals (Browning 1967). From reactivity as well as soil additive perspectives, these components play a significant role. Ash is an important parameter for oxidative ammonolysis because the inorganic components of the ash such as sodium carbonate and potassium carbonate can act as catalysts for hydrolysis of the lignin macromolecules during oxidative ammonolysis (Demirbas 2000). From the soil additive perspective, the inorganic components of the ash have a fertilising effect of their own (Meier et al. 1994). Wood ash components temporarily raises soil alkalinity, making potassium and phosphate more available to the crop (depending on the actuall pH range) (Sharland 1997).

Elemental analysis is probably the most commonly used method in the characterization of lignin and similar carbonaceous materials and provides information on the distribution of major elements. Technical lignins contain carbon and oxygen as major elements, in addition to hydrogen, nitrogen and sulphur. The carbon content varies from approximately 40 to 60% and oxygen content from 30 to 50%. Both carbon and oxygen can give information on the state of degradation of the technical lignin by isolation processes employed. Nitrogen and sulphur contents are much lower, varying from 1 to 4% and 0 to 2% respectively.

As most of the pulping methods used to separate lignin from cellulose contain sulphur compounds, sulphur containing groups such as thiols and sulphonic acid groups introduced as a consequence of sulphide or bisulphite pulping have a particular significance especially the sulphonic group (in lignosulphonates, most of the sulphur is located in sulphonic acid groups) as they direct the reactivity of the technical lignin macromolecule. The high polarity of the sulphonic acid group makes the technical lignin

macromolecule water soluble which is often disadvantageous in attempts to achieve modification in non aqueous media (Sarkanen and Ludwig 1971).

The proportions of different functional groups such as methoxyl, hydroxyl, carbonyl and carboxyl groups are also important indicators as to which extent had the lignins been modified during isolation (Dence and Lin 1992). They are also indicators of the purity of such isolated lignins as lignins often contain admixtures of non-methoxylating substances, in particular carbohydrates. When such isolated lignins are applied, such as in oxidative ammonolysis, the amount of affixed groups and molecules (nitrogen in the case of oxidative ammonolysis) can be assessed by means of the decrease in the methoxyl group content. In fact, kinetic studies on oxidative ammonolysis revealed that there is a direct relationship between nitrogen incorporation and the decrease of methoxy groups (Schiene 1994).

A quantitative measurement of carboxyl groups is also important as it provides information regarding the degree to which the lignin has been degraded or otherwise modified as a result of a chemical or biological treatment (Dence and Lin 1992). In general, when native lignin is subjected to chemical (e.g. pulping and bleaching) or biological treatments, significant amounts of carboxyl groups are found. This effect is particularly pronounced in oxidative treatments where the resulting rupture of the phenolic rings in the lignin gives rise to carboxyl containing entities (Dence 1992).

It is well known that the chemical reactivity of lignin polymers is strongly influenced by their content of hydroxyl groups because it is through ionisation of this functional group that many lignin reactions takes place. Among the criteria for selecting raw materials for oxidative ammonolysis (technical lignins and similar carbonaceous materials) is that they should have a certain content of phenolic moieties whose unsaturated *pi* electronic structure is essential in the modification of ligneous raw materials into nitrogen-enriched humus-like products. Technical lignin types can be characterised by the phenolic functionality and many lignin modifications are based on the phenolic character, a property that is not easily quantified in a complex material. The content of phenolic

hydroxyl groups is of interest also regarding pulping and bleaching processes, as they increase the hydrophilicity of residual lignin. They are also reactive towards many delignification agents e.g. oxygen and chlorine dioxide (Tiainen et al. 1999).
Other groups such as the carbonyl groups in lignins are also of interest to assess the modifications which occur in the lignin polymer during pulping, bleaching or ageing of wood and pulps (Sevillano et al. 2001).

Physiological active substances which have negative effects on the growth and development of plants are sometimes present in the parent materials (Fiedler 1979). These result from the presence of a high proportion of reducing substances such as carbohydrates in the raw materials. In general, glucose is known to form nitrogen containing heterocyclic compounds mainly imidazoles and pyrazines when reacted with ammonia (Kort 1970). It is in this regard that the content of reducing sugars present in the technical lignin is also a very important criterion for the selection of raw materials for oxidative ammonolysis.

The selected feed stocks were characterised for physical and chemical properties in order to determine the most suitable route for the manufacture high grade humic materials. The results were compared to values obtained in literature.

4.2 Materials

- A bagasse lignocellulosic residue i.e. bagasse partially stripped from sugars for furfural production process (Illovo Sugar Ltd., South Africa).
- A bagasse lignin, trade named Sucrolin, which is produced through solvent extraction of bagasse with weak alkali (Illovo Sugar Ltd., South Africa).
- A calcium lignosulphonate sample (LignoTech, South Africa).
- A Kraft lignin, Indulin AT, obtained from Westvaco Co., USA, was used for comparison.

4.3 Methods

4.3.1 Ultimate analysis

The **moisture and ash contents** were determined according to TAPPI standard method T12wd-82 and T 211 om-85 respectively. The **elemental composition** was determined using CNS analyser as described in Tyhoda (2003).

4.3.2 Functional group analysis

4.3.2.1 Methoxyl group

The methoxyl group contents were determined according to a method originally developed by Zeisel and co-workers and later modified by Vieböck and Schwappach into what is now known as the Zeisel-Vieböck Schwappach method. This method, which is generally considered universal, is based on the following quantitative reaction equations:

$$R\text{-}OCH_3 + HI \rightarrow R\text{-}OH + CH_3I \qquad (4\text{-}1)$$

$$CH_3I + Br_2 \rightarrow CH_3Br + IBr \qquad (4\text{-}2)$$

$$IBr + 2Br_2 + 3H_2O \rightarrow HIO_3 + 5HBr \qquad (4\text{-}3)$$

$$HIO_3 + 5HI \rightarrow 3I_2 + 3H_2O \qquad (4\text{-}4)$$

$$3I_2 + 6Na_2S_2O_3 \rightarrow 6NaI + 3Na_2S_4O_6 \qquad (4\text{-}5)$$

In general, when a compound is heated with concentrated hydriodic acid, the alkoxy group is cleaved and converted to a corresponding alkyl iodide. The methyl or ethyl iodide in the carrier gas is collected in a trap containing a solution of sodium acetate in glacial acetic acid to which is added a few drops of bromine. The bromine reacts with the volatile alkyl iodide to produce the corresponding alkyl bromide (RBr) and iodine bromide (IBr). The excess bromine further oxidises the iodine bromide to iodic acid (HIO_3). The remaining bromide is then reduced by formic acid (HCOOH) to hydrogen bromide which is neutralized by the sodium acetate solution. Finally, the iodine thus produced is titrated with a dilute standard sodium thiosulphate solution using 1% starch solution as an indicator. Since six atoms of iodine are liberated for each alkoxyl group, the alkoxy content can be quantitatively determined with very high accuracy even on a micro scale (Chen 1992).

This method for determination of methoxyl groups has been continuously improved, and today almost all research laboratories have their own modifications, varying mainly by facilities available and purification procedures for CH_3I from I_2 and HI impurities. For our purpose, the analysis was carried out as described by Zakis (1994).

4.3.2.2 Carboxyl group

The carboxyl group contents were determined according to the potassium iodide/potassium iodate method. The analysis was carried out by weighing 500mg into a 200ml flask followed by the sequential addition of 5ml ethanol, 30ml of 0.1N $Na_2S_2O_3$ solution, 25ml 0.1 N KI/KIO_3 solution and 5ml 5% $CaCl_2$ solution. A standard was also prepared in the same way only without KI/KIO_3 solution. The samples were then carefully closed and kept in the dark for 8 days at room temperature. After 8 days, they were washed with deionised water over a G4 fritted filtering glass. The filtrate was titrated with excess $Na_2S_2O_3$ solution. The carboxyl group content was determined according to equation 4-6 below.

$$\%COOH = \frac{(V_B - V) \times t \times 4.5}{E} \qquad (4\text{-}6)$$

where V_B is the amount of KI/KIO_3 used in the standard

V is the amount of KI/KIO_3 used in the sample

t is the normality of the KI/KIO_3 solution

E is the mass of the material

4.3.2.3 Hydroxyl group

The hydroxyl group content was determined as described by Månsson (1983). The method comprised acetylation, selective aminolysis of the phenolic groups by pyrrolidine (aminolysis), and the determination of the resulting 1-acetylpyrolidine by gas chromatography.

Acetylation

Acetylation was carried out as described by Glasser (1993) with a slight modification. 500mg of material was dissolved with 10ml of pyridine before an equal amount of acetic anhydride was added. The reaction carried out at room temperature with continuous stirring under flowing nitrogen for 24 hours. The acetylated lignins samples were recovered by precipitating the reaction mixture with 100ml of 0.1N HCl. The acetylated lignin samples were washed several times with 0.1N HCl to remove the pyridine. This was followed by washing with distilled water to remove the HCl from the lignin derivatives. The derivatives were collected and freeze dried for 24 hours and stored in a desiccator.

Determination of the total OH groups by acid hydrolysis of the acetylated samples

4ml of 50% sulphuric acid was added to 60mg of each of the acetylated lignin samples. The mixture was refluxed under nitrogen for 1 hour. 10ml of water was added drop wise and the refluxing was carried out for a further 10min. The formed acetic acid (see reaction scheme presented in figure 4-1 below) was then distilled by a drop wise addition of water (ca. 30ml).

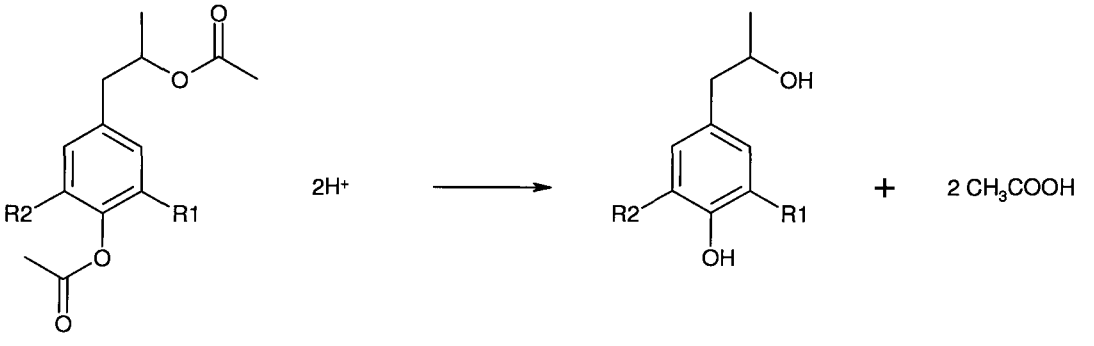

Figure 4-1: Acid hydrolysis of an acetylated lignin group.

The distillate was standardised with barium chloride in the absence of sulphuric acid. The acetic acid was determined through by potentiometric titration with 0.1N sodium hydroxide. The total hydroxyl group content was determined according to equation 4-7 below.

$$\%OH_{tot} = \frac{f\ a \times 170}{E\text{-}\ 4.2 \times f \times a} \qquad (4\text{-}7)$$

f = correction factor for the 0.1N sodium hydroxide

a = volume of sodium hydroxide used (ml)

E = mass of the acetylated sample in (mg)

Determination of the free phenolic OH group through amminolysis

10mg of the each of the acetylated lignin samples was suspended in 1ml dioxane. The aminolysis was started by adding 1ml of pyrrolidine containing a known amount of internal standard 1-proponylpyrollidine. In a time interval from 5 and after 10 minutes respectively, 0.5µl of sample was injected in GC chromatograph (in total seven measurements). The calibration of the GC method was performed through the aminolysis of acetylvanillin and acetoxybenzoacetic methyl ester. The curve began quite steeply (see top part of the reaction scheme presented in figure 4-2 below) and this part represented the formation of the two esters. After 10 minutes, fast aminolysis of the phenol ester was completed and the curve flattened in accordance with the slow formation of the aliphatic ester (see lower part of the reaction scheme presented in figure 4-2 below). The hydroxyl concentration was then determined by extrapolation of linear part of the curve towards zero.

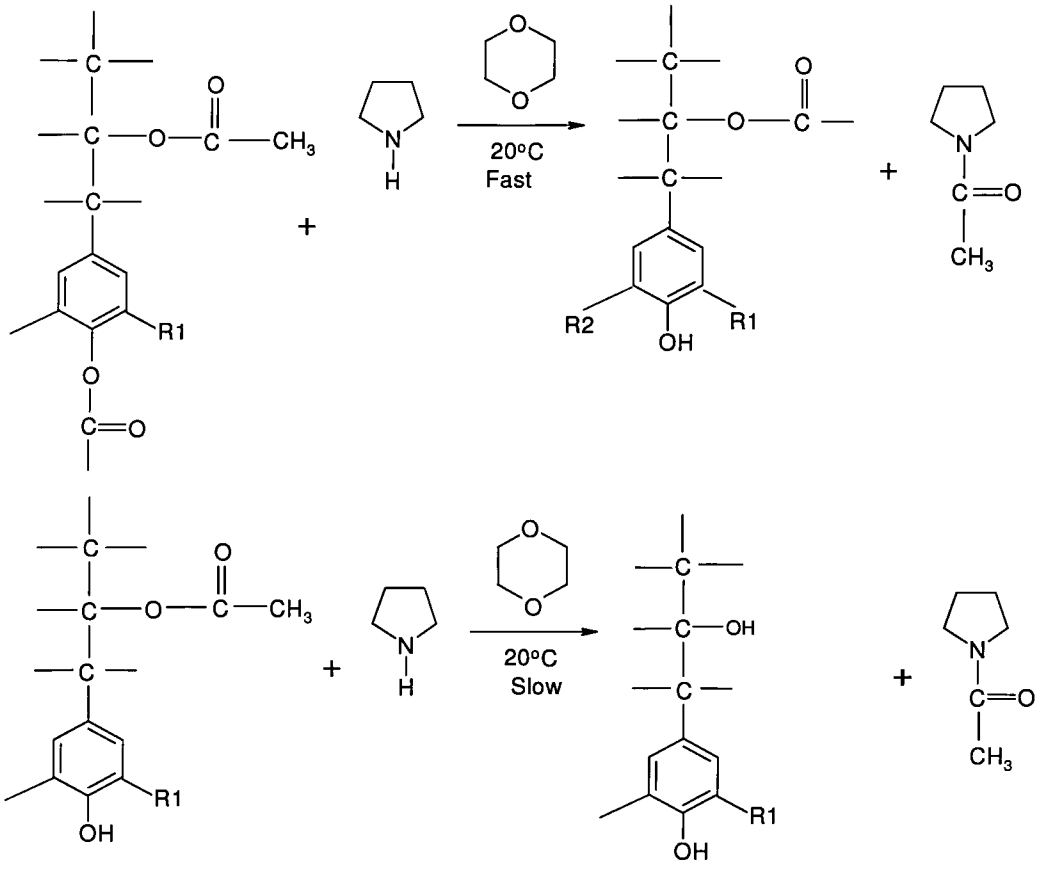

Figure 4-2: Aminolysis of acetylated free phenolic groups

4.3.2.4 Carbonyl group

The carbonyl group (CO) content was determined by an oximation method as described in Zakis (1994). Using this method, about 200mg of the lignin was placed into a titration cell and dissolved in 20mg of ethanol. The pH of the solution was adjusted to 4. The temperature of the titration cell was adjusted and maintained at 25°C. 138mg (2mmol) of $NH_2OH{\cdot}HCl$ in 40ml of 80% ethanol was added. Nitrogen saturated with ethanol vapours was passed through the solution at 25°C. A magnetic stirrer was turned on and the HCl released in the course of oximation was back titrated with 0.1 N NaOH with an automatic autotitrator which was adjusted to pH 4. A curve of volume of 0.01 N NaOH was made after certain intervals of time. The test was stopped when the pH did not change when more NaOH was added. The concentration of the CO-group contents was calculated according to equation 4-8 below.

$$\%CO_{tot} = \frac{a \times f \times 0.2801}{A} \times 100 \qquad (4\text{-}8)$$

Where

a = a volume of 0.01 N NaOH used for the titration

f = titer of 0.01 N NaOH

0.2801 = a mass of CO-groups, equivalent to 1ml of 0.01 N NaOH, in mg

A = lignin mass in mg

4.3.3 *Analysis of reducing substances*

A modified method originally developed by Bertrand (1906) based on the use of the [2]Fehling solution was employed for the reducing sugar determination.

Principle: According to Keusch (2003), Fehling's tests take advantage of the ready reactivity of aldehydes by using the weak oxidizing agent, cupric ion (Cu^{2+}), in alkaline solutions. In addition to the copper ion, Fehling's solution contains a tartrate ion as a complexing agent to keep the copper ion (Cu^{2+}) in solution. Without the tartrate ions, cupric hydroxide would precipitate from the basic solution. The tartrate ion is unable to complex cuprous ion Cu^{+}. Therefore the reduction of Cu^{2+} to Cu^{+} by reducing sugars results in the formation of an orange to red precipitate of Cu_2O.

The reducing substances are quantitatively determined by adding excess Fehling solution to the sample containing the reducing substances. The amount of Fehling that has been reduced to copper (I) oxide is then determined in a stoichiometric reaction (see equation below) by the addition of ferric (III) sulphate to the mixture. This is done in by acidifying with H_2SO_4. Under these conditions, the ferric (III) sulphate is reduced to ferric (II) sulphate. The amount of ferric (II) sulphate formed can be determined by back titration with $KMnO_4$.

$$Cu_2O + Fe_2(SO_4)_3 + H_2SO_4 \rightarrow 2CuSO_4 + 2FeSO_4 + H_2O \qquad (4\text{-}9)$$

$$10FeSO_4 + 2KMnO_4 + 8H_2SO_4 \rightarrow 5Fe_2(SO_4)_3 + K_2SO_4 + 2MnSO_4 + 8H_2O \qquad (4\text{-}10)$$

[2] A Fehling's solution is a solution of $CuSO_4$ / tartrate.

Experimental: 5g of each of the material was dissolved in 100ml–deionised water to make up a 5% solution. 10ml of the solution was added into a 250ml Erlenmeyer flask. 20ml of Fehling I and II reagents were added. Boiling stones from a magnesia rod were added and the solution was moderately boiled for 3 minutes (counting from the time boiling was first observed). It was allowed to cool for 15 minutes and filtered under reduced pressure into a vacuum flask using a G4 fritted filtering glass. The precipitate in the Erlenmeyer flask was washed with water. It was allowed to settle for 15min, after which the water was carefully decanted into the fritted glass filter. The fritted glass filter was also washed immediately because the red copper (I) oxide becomes difficult to dissolve with time. 2 drops of KMnO4 were added to a 15ml solution of iron sulphate. This solution was added to the precipitate in the Erlenmeyer flask forming a green solution. The fritted glass filter was connected to a new vacuum flask. The green solution from the Erlenmeyer flask was added to the fritted glass filter. The fritted glass filter was washed with a fresh solution of iron sulphate. The fritted glass filter was washed with deionised water 3–4 times. The red copper (I) oxide was then back titrated with a 0.1M solution of $KMnO_4$. The amount of reducing substances is determined as follows: From the table (see appendix D), the amount of reducing sugar corresponding to the amount of $KMnO_4$ used was determined.

Calculations: The amount of reducing substances was determined using equations 4-11 and 4-12 depending on the amount of Fehling's solution used i.e. if 10ml of each of the Fehling solution was used, equation 4-11 was used, whereas if 20ml is used, equation 4-12 was used.

$$\%RS = \frac{Sugar(mg)}{10} \qquad (4\text{-}11)$$

$$\%RS = \frac{Sugar(mg)}{5} \qquad (4\text{-}12)$$

From the table, (see appendix 4-A), the mass of sugar in mg could be determined by the corresponding value of n/10 permanganate solution.

4.3.4 Determination of the sugar components from the lignocellulosic residue

As discussed above, the monosaccharide sugar components are an important parameter for oxidative ammonolysis of technical lignins. To characterise the monosaccharide component of this material, the lignin component had to be completely removed to protect the HPLC columns. This was done using a method first pioneered by (Klauditz 1975). This method is based on the use of chlorine dioxide in the presence of acetic acid. Chlorine dioxide finds wide application in pulp bleaching because of its selectivity. It acts as an electrophile and selectively degrades aromatic rings with free hydroxyl groups. It reacts very slowly with carbohydrates and hence its use to prevent the degradation of the polysaccharide units (Sjöström 1993). The lignin free material was then hydrolysed under mild conditions using trifluoroacetic acid (TFA) in preparation for analysis by HPLC. Water and alkali soluble polysaccharides can be hydrolyzed by TFA. Cellulose and cellulose containing substances are dissolved in concentrated TFA and subsequently hydrolyzed in homogenous reaction. The hydrolysis takes one hour and the acid is completely removed by evaporation (Fengel et al. 1977). For our purpose, both gentle and total hydrolysis were employed because then, through gentle hydrolysis, the different types of sugars could be determined of which xylose was of main interest, and with total hydrolysis the total amount of glucose could be determined.

Experimental

Pre-treatment: Residues of resins were removed from the sample through extraction for six hours with acetone using a Soxhlet extraction apparatus. The samples were dried afterwards in air under a fume hood.

Hydrolysis: Total hydrolysis was carried out to hydrolyse all cellulose such that the total amount of glucose could be determined. A mild hydrolysis was used for the determination of the other monosaccharides such as xylose.

Total hydrolysis: Ca. 20 mg of the sample was weighted into a round bottom flask (50ml; narrow neck) and closed with a ground glass stopper. The moisture content of the sample was determined according to TAPPI standard method T12wd-82. The residual moisture

from the sample was removed under vacuum by placing the sample into a desiccator which contained phosphorus pentoxide as a drying agent. After drying, 3ml of water-free trifluoroacetic acid (Merck) was added to the sample and the flask closed with a stopper. The sample was allowed to swell for at least 40 hours and then was refluxed at 105°C for 1 hour. The TFA was diluted further to a concentration of 80% by volume by adding 1.054ml of water and refluxed for another 35 min. The TFA was diluted to a concentration of 30% by volume by adding 9.153ml of water and refluxing for another 2h to complete the hydrolysis step. The hydrolysate was washed with water until the formed ester was saponified and the distillate contained no acids. This was especially important to protect the lead coated column material of the HPLC. The washing procedure was carried out under vacuum in a rotor vapour at 40°C. Washing with water (~10ml) had to be repeated 12 to 15 times with the last washing done to dryness. The hydrolysed sugars were dissolved in 2ml of water and filtered through a 45µ analytical filter. This solution was then analysed by HPLC.

Mild hydrolysis: Mild hydrolysis was used in the analysis of non-glucose units which could be degraded at high acid concentrations. In principle, the hydrolysis was carried out in the same way as a total hydrolysis. Only the first step i.e. the refluxing for 1h at 105°C was excluded in this procedure. After swelling, at first the first amount of water was added so that the procedure of hydrolysis started with a TFA concentration of 80%.

Analysis of the sugar monomers: The sugar monomers were distributed with water on a REPRO- GEL PB (300mm x 8mm ID) column at an elution rate of 0.5ml/min using a temperature of 60°C in the column oven. Detection was carried out using an LC – MSD system. Calibrations were done by preparing standards of the corresponding monomers.

4.4 Results and discussion

4.4.1 Ultimate analysis

Ash and dry matter composition: The compositions of the technical lignins are presented in table 4-1. The dry matter of the technical lignins varied between 93 and 97%. The ash contents of the materials ranged between 2.7 and 21.30%. The variation in

the ash contents was quite wide but typical of the various pulping processes with which materials were isolated. The ash contents of the Sucrolin and Indulin AT were distinctly lower than those of the lignosulphonate and the lignocellulosic residue. In general, Indulin AT is a purified form of Kraft pine lignin while Sucrolin is a lignin recovered from a steam explosion process. Technical lignins recovered from that process have a low content of contaminants such as ash and sugars (Lora and Glasser 2002). The high ash content of the calcium lignosulphonate was characteristic of the sulphite pulping process. (Meier et al. 1994) found 23% ash in lignosulphonate from LignoTech. Lignosulphonates of up to 34% ash have also been found (Nadif et al. 2002).

Elemental composition: The carbon content varied between 45 and about 63%. The low carbon content of the lignosulphonate was typical of the sulphite pulping process. The high C/N ratios were typical of lignins, which is basically why these materials cannot be used as soil improvement agents without nitrogen modification. The C/N ratio for plant nutrient materials should not be more than 20. The carbon contents of the Sucrolin and the Indulin AT were in agreement with findings from other studies (Reimann 1990; Jakab et al. 1995).

Table 4-1: Elemental composition of the raw materials (values given on oven dry matter basis).

Raw material	Dry Matter [%]	Ash [%]	elemental composition [%]			C/N Ratio
			N_{total}	C_{total}	S_{total}	
Lignosulphonate	94.94	21.30	0.14	45.2	4.93	322.9
Lignocell. residue	95.70	11.90	0.43	51.3	0.11	119.3
Sucrolin	95.63	2.70	0.63	64.2	0.19	101.9
Indulin AT™	97.70	3.0	1.17	62.9	2.11	53.8

The carbon content of the bagasse lignocellulosic material was lower than the Indulin AT and the Sucrolin which could be expected due to the cellulosic content of the material. The sulphur contents among the technical lignins varied widely but the contents were

typical of the various processes with which they were isolated. The calcium lignosulphonate had high sulphur content which as mentioned, is typical of the bisulphite pulping process. The low sulphur content of the Sucrolin could be expected as previous studies have also shown that it is typical for materials from biomass technologies (Lora and Glasser 2002).

4.4.2 *Functional group analysis*

The methoxyl group contents of the materials ranged from 4 to 12.8% (table 4-2). The lowest value was obtained with the lignocellulosic residue which was characteristic of the cellulosic content of the material which was about 39%.

Table 4-2: Comparison of the functional group contents of the technical lignins (values given on dry matter basis).

Raw material	functional groups [%]					
				OH		
	OCH_3	COOH	CO	total	Phenolic	Aliphatic
Lignosulphonate	9.3	3.5	--	--	--	--
Lignocell. residue	4.0	2.7	6.5	6.8	1.5	5.3
Sucrolin	8.3	7.6	4.9	7.5	4.7	2.8
Indulin AT	12.8	5.4	1.6	12.8	6.3	6.5

The Indulin AT had the highest methoxyl group content in agreement with findings by Jakab et al. (1995), showing that this material was highly condensed which has also been reported in other works (Giroux et al. 1988). On the contrary, by virtue of the Sucrolin being a grass lignin, it had distinctly lower methoxyl group content than Indulin AT which is a *Gymnosperm* lignin. The low methoxyl group content of the Sucrolin resulted from the degradation of the lignin due to the acid hydrolysis process used to prepare furfural from the bagasse. The value obtained was in agreement with values obtained in literature (Reimann 1990; Jakab et al. 1995).

The carboxyl contents of the materials ranged form 3.5% to 7.6%. The high carboxyl group content of the Sucrolin indicates that this technical lignin was highly oxidised due to the autohydrolysis reaction during the furfural reaction. This bodes well for any oxidative treatment such as the oxidative ammonolysis reaction as oxidation is central to the success of the modification of such materials.

The carbonyl contents varied between 1.6 and 6.5%. While the Sucrolin had a higher CO group content than the Indulin AT due to the fact that it was a grass lignin, the higher CO group content of the lignocellulosic residue was also due to the contribution from the cellulosic part of the material.

The hydroxyl group contents

The hydroxyl group content of the Indulin AT was higher than that of the Sucrolin and the lignocellulosic residue. This was more so for the lignocellulosic residue because of the considerable cellulosic content. The phenolic hydroxyl group of the Sucrolin was 62.7% per 100 units of phenylpropane compared to 37.3 aliphatic. The Indulin AT had a rather high content of aliphatic hydroxyl content implying that the technical lignin was rich in condensed-type linkages of lignin.

4.4.3 Reducing substances

Five membered, nitrogen containing heterocyclic compounds, (mainly imidazoles and pyrazines) have a potential of causing growth inhibiting or phytotoxic effects on crops. These are formed as a result of a high proportion of reducing substances in the raw materials (especially five membered ring monosaccharide sugars like xylose). Figure 4-3 below shows the proportion of reducing substances of the three raw materials in percentage. The relatively high content of reducing substances in the calcium lignosulphonate materials was quite remarkable compared to the other two materials.

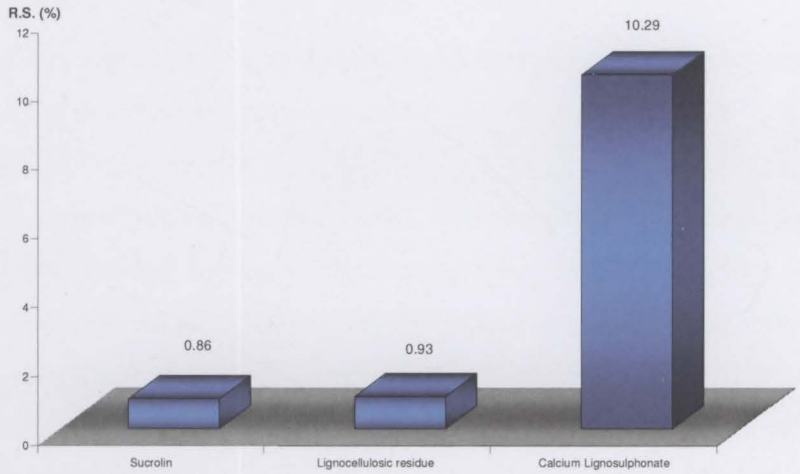

Figure 4-3: Reducing sugar contents of the three technical lignins.

4.4.4 The monosaccharide components of the lignocellulosic residue

Table 4-3 below presents the carbohydrate composition of the lignocellulosic residue. The total carbohydrate content was determined by Curie-Point Pyrolysis GC/MS (see chapter 3). As the results show, a huge bulk of this component was glucose 87.2% and the xylose; the component of major interest was a negligible 0.65%.

Table 4-3: Total carbohydrate content of lignocellulosic bagasse residue and monosaccharide composition of the delignified material.

total carbohydrates determined by Curie-point pyrolysis [%]	monosaccharide content [%]				
	Glucose	mannose	fructose	xylose	arabinose
38.9	87.2	<0.31	<0.45	0.65	0.02

At such low concentration, the xylose was deemed not to be a concern as far as phytotoxicity of the products was concerned. In addition, since the oxidative ammonolysis reaction took place at 70°C, it was not expected that these conditions could solubilise the cellulosic component into the individual components shown in the table.

4.5 Conclusion

The quality of the technical lignins i.e. Sucrolin and the calcium lignosulphonate differed mostly as determined by the isolation procedure and plant origin. The lignocellulosic residue, as expected, had a higher cellulosic content. Although some of the properties were expected, they were also expected to have fundamental influences on the behaviour of the raw materials during the oxidative ammonolysis reaction, and the products so obtained. Regarding the reaction process, the following postulations were made:

- a high content of carboxyl group content as found in the Sucrolin could result in the formation of high amounts of foam, necessitating the use of an anti foaming agent or down regulation of air or oxygen which in turn could result in lower N content in the products.
- the calcium lignosulphonate was water-soluble as a result of its high content of sulphonic groups. The sulphonic groups were also responsible for directing the reactivity of this material.

Regarding the products, the following postulations were made:

- the calcium lignosulphonate had a high reducing content substances which could have negative implications on the products that could be obtained from the material as a result of the formation of substances that could render the material toxic to plants.
- the high content of carboxyl group of the Sucrolin meant that the material was highly oxidised meaning that a higher N-content could be expected in the products if the foam could be controlled.
- the high cellulosic content of the bagasse lignocellulosic residue was not expected to have any negative influence on the products as the oxidative ammonolysis uses milder reaction conditions and hence it could not be expected that the cellulosic material could be solubilised into individual components.

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4.7 Appendices

Appendix 4-A: Table for reducing sugar determination after titration according to Bertrand (1906).

0,1 N $KMnO_4$-Lösung in ml	Zucker in mg	0,1 N $KMnO_4$-Lösung in ml	Zucker in mg	0,1 N $KMnO_4$-Lösung in ml	Zucker in mg
3,2	9,80	8,2	26,05	13,2	43,25
3,3	10,10	8,3	26,38	13,3	43,61
3,4	10,43	8,4	26,72	13,4	43,96
3,5	10,75	8,5	27,05	13,5	44,32
3,6	11,07	8,6	27,39	13,6	44,68
3,7	11,39	8,7	27,72	13,7	45,03
3,8	11,71	8,8	28,06	13,8	45,39
3,9	12,03	8,9	28,39	13,9	45,75
4,0	12,35	9,0	28,72	14,0	46,10
4,1	12,67	9,1	29,06	14,1	46,45
4,2	12,99	9,2	29,40	14,2	46,81
4,3	13,30	9,3	29,74	14,3	47,16
4,4	13,63	9,4	30,08	14,4	47,52
4,5	13,95	9,5	30,42	14,5	47,87
4,6	14,26	9,6	30,76	14,6	48,23
4,7	14,58	9,7	31,10	14,7	48,58
4,8	14,91	9,8	31,44	14,8	48,94
4,9	15,23	9,9	31,78	14,9	49,26
5,0	15,55	10,0	32,11	15,0	49,66
5,1	15,86	10,1	32,45	15,1	50,02
5,2	16,18	10,2	32,80	15,2	50,39
5,3	16,50	10,3	33,14	15,3	50,75
5,4	16,82	10,4	33,49	15,4	51,12
5,5	17,14	10,5	33,83	15,5	51,48
5,6	17,46	10,6	34,18	15,6	51,85
5,7	17,78	10,7	34,52	15,7	52,21
5,8	18,11	10,8	34,87	15,8	52,58
5,9	18,43	10,9	35,21	15,9	52,94
6,0	18,76	11,0	35,56	16,0	53,30
6,1	19,08	11,1	35,90	16,1	53,67
6,2	19,41	11,2	36,24	16,2	54,04
6,3	19,73	11,3	36,58	16,3	54,41
6,4	20,06	11,4	36,92	16,4	54,78
6,5	20,38	11,5	37,26	16,5	55,16
6,6	20,71	11,6	37,60	16,6	55,53
6,7	21,03	11,7	37,94	16,7	55,90
6,8	21,36	11,8	38,28	16,8	56,27
6,9	21,68	11,9	38,62	16,9	56,65
7,0	22,02	12,0	38,96	17,0	57,02
7,1	22,35	12,1	39,32	17,1	57,39
7,2	22,69	12,2	39,68	17,2	57,76
7,3	23,02	12,3	40,04	17,3	58,13
7,4	23,36	12,4	40,39	17,4	58,50
7,5	23,69	12,5	40,75	17,5	58,87
7,6	24,03	12,6	41,11	17,6	59,24
7,7	24,36	12,7	41,47	17,7	59,61
7,8	24,70	12,8	41,83	17,8	59,98
7,9	25,04	12,9	42,18	17,9	60,34
8,0	25,38	13,0	42,53	18,0	60,70
8,1	25,71	13,1	42,89		

5.0 Oxidative ammonolysis of South African technical lignins and chemical characterisation of the *N*-lignins

Abstract

South African technical lignins derived from the sulphite pulping process (a calcium lignosulphonate), sugar cane bagasse (Sucrolin), and a partially stripped sugar cane bagasse (a lignocellulosic residue with a low lignin content) were N-modified according to a patented technology based on oxidative ammonolysis used in the manufacture of NOVIHUM™, a high grade humic material made from lignite and similar carbonaceous materials. A Kraft lignin, Indulin AT, was used for comparison. Elemental analysis of the products revealed nitrogen contents of the modified products in the following order: N-Sucrolin (6.25% N), N-calcium lignosulphonate (3.36% N), N-Indulin AT (3.07% N) and the N-modified bagasse lignocellulosic residue (2.64% N). The values obtained were within the range found in *N*-lignins reported in literature, confirming the feasibility of using these raw materials. The total nitrogen of the bagasse lignocellulosic residue was increased to 3.59% after pre-treatment with hydrogen peroxide and oxidative ammonolysis in oxygen instead of air. Changes in molecular structure of the N-lignins during oxidative ammonolysis were monitored by Curie-Point Pyrolysis GCMS and ^{31}P NMR spectroscopy. A combination of analytical techniques including Curie-Point Pyrolysis GCMS, X-ray photoelectron spectroscopy (XPS) as well as ^{15}N CPMAS spectroscopy gave new insights into the nature of N-binding forms of N containing compounds in N-lignins.

5.1 Background

The modification of technical lignins to make humic materials can be achieved using a reaction process called oxidative ammonolysis or ammoxidation. Through this process, technical lignin macromolecules are oxidised by anionic oxygen radicals generated in

an alkaline medium and subsequent reaction of the products with ammonia. This complex reaction sequence includes demethoxylation, demethylation, formation of quinones, oxidation of aliphatic side chains, cleavage of aromatic ring units and nitrogen enrichment in a similar way to the natural humification processes. Oxidative ammonolysis was earlier performed with oxygen under increased pressure. However, due to disadvantages associated with products made under these conditions, this process is now performed exclusively under normal pressure (Fischer and Schiene 2002). The humic products obtained from this process are trade named NOVIHUM™. NOVIHUM™ has unique properties as a high grade humus and long-lasting, organo-mineral nitrogen fertiliser. The technical procedure developed for the production of NOVIHUM™ enables the specific synthesis of products in which nitrogen is bonded to the lignin in different forms e.g. some nitrogen can be slowly mineralised and made available to plants over extended periods of time. Studies by workers in this field have shown that *N*-modified technical lignins had a positive effect on crops (Ramirez et al. 1997; Ramirez-Cano et al. 2001). A pilot plant was commissioned in 2000, in Freienhufen Germany, to produce NOVIHUM™ in sufficient quantities for field trials. NOVIHUM™ has since 2000 been successfully tested in rehabilitation activities and initial greening of problematic sites around the world (Liebner et al. 2006).

The products obtained are called *N*-modified lignins or *N*-lignins. The technical procedure developed for the production of *N*-lignins enables the specific synthesis of products with nitrogen which is differentially bonded to the lignin or lignin based polymer and hence released to plants over extended periods of time. The following classification is used to designate different forms of nitrogen bonded to the lignin macromolecule as found in *N*-lignins (Wang et al. 1964; Schiene et al. 1979):

- ammonium nitrogen (available to plants immediately),
- amide nitrogen (available to plants the over the mid-term), and
- strongly bonded nitrogen (available to plants over a longer period).

The stepwise mineralisation of the nitrogen from *N*-lignins is a unique and a singly most important feature of these materials as it results in the reduction of nitrogen leaching

into the environment in the form of nitrates. This is sometimes the case with easily soluble inorganic fertilisers, mainly as a result of over application. In addition, plants can receive a constant supply of nitrogen over several growing periods without additional inputs.

At the Institute of Plant and Wood Chemistry of the Technical University of Dresden, Germany, the process of oxidative ammonolysis has been extensively studied since the 1970s. Intensive research on different process conditions and materials from different sources has been undertaken, leading to a patent of the technology being obtained for the process (Fischer et al. 1993) , as well as the novel *N*-modified products developed from it, trade named NOVIHUM™ (Fischer et al. 2000). A review of this work has been given by Fischer and Schiene (2002). Earlier, Meier et al. (1994) had also given a review of the literature covering the development of the oxidative ammonolysis procedure from 1973 to 1993. Recently, Liebner et al. (2006) has reviewed pot and field trials applications of NOVIHUM™ under various soil and climatic conditions in various parts of the world.

Investigations of N-lignins, in particular their nitrogen containing units, is of current interest in association with key structures responsible for plant growth (Potthast et al. 1996). Studies on products obtained from the high-pressure technology using lignin model substances aimed at the determination of nitrogen containing compounds related to lignin as well as possible pathways for their formation, using ^{15}N-NMR, infra red (IR) spectroscopy as well as GCMS, revealed among the substructures identified, aromatic nitriles, low molecular weight compounds with ring bonded nitrogen as well as certain amides (Potthast et al. 1996). N-heterocyclic compounds were not found.

Although these studies were based on lignin model substances, the results have shown that solid-state NMR, especially ^{15}N CPMAS NMR spectroscopy can generally contribute to a certain extent in the elucidation of the nature of N-binding compounds in ammonoxidized lignins. One of the most critical requirements for obtaining meaningfull results with ^{15}N CPMAS NMR is that there should be a comparatively high

N content in the samples. Sensitivity can also be improved by ammoxidation of the samples using ^{15}N labelled ammonia. This is done to reduce the effect of the unfavourable gyromagnetic ratio as well as the low natural occurrence of the ^{15}N nucleus.

One of the most convenient and frequently used methods in the characterisation of technical lignins and similar ligneous materials is the Curie-Point Pyrolysis GC/MS. It is a simple technique which does not require pre-treatment and derivatisation of the compounds. This method employs standardized thermo-chemical degradation processes. The degradation processes result in the fragmentation of the polymers. The structure of the polymers can be deduced from the fragments so formed. Pyrolysis-GC/MS, therefore, belongs to deductive methods for structural analysis (Roschy and Liebner 2002).

As there is no single analytical technique which can lead to a full characterisation of technical lignins, *N*-lignins and similar materials, a combination of different techniques has to be used. X-ray photoelectron spectroscopy (XPS) is another useful tool in the elucidation of lignins, especially lignin contents on the surface of pulp fibre (Johansson et al. 1999). XPS works by irradiating the sample material with monoenergetic soft x-rays causing electrons to be ejected. Identification of the elements in the sample can be made directly from the kinetic energies of these ejected photoelectrons. The relative concentrations of elements can be determined from the photoelectron intensities. XPS can also be used to analyze the change in surface chemistry of a material after chemical or physical treatments. XPS measurement of nitrogen signals can be used in to monitor the incorporation of nitrogen and its distribution in different N-containing groups within the lignin macromolecules. However, surface analyses may be unreliable due to surface contamination. Hence a complimentary technique such as NMR spectroscopy should be used. Chemical forms often give distinct results in the XPS, which are analogous to the chemical shift in NMR (Lambert et al. 1999).

In South Africa, initial studies in the field of oxidative ammonolysis of technical lignins were conducted with the aim of establishing the potential for using a selection of locally produced technical lignins and local lignite deposits for the manufacture of high grade, slow nitrogen releasing humic materials (Tyhoda 2003). These screening studies i.e. determine the reactivity of the selected materials in terms of nitrogen incorporation were carried out using the high pressure process. The selected materials were compared with German obtained raw materials which were used in the manufacture of NOVIHUM™ in terms of the following:

- chemical and physical properties
- whether pre-oxidation of the raw materials prior to oxidative ammonolysis could be used to increase the total nitrogen that could be incorporated
- total nitrogen that they could incorporate as well as the different forms in which the nitrogen was bonded to the lignin macromolecule.

The results indicated that the quality of the selected South African lignites was very low due to high ash contents (about 50%) as compared to the German obtained lignites (5%). The South African technical lignins were of better quality, and showed huge potential with up to 10% N compared to 5% of the NOVIHUM™. Their nitrogen content could be further increased to 12.7% with hydrogen peroxide pre-treatment.

Ninneman (2002) has since developed an experimental, small scale, laboratory plant using the ambient pressure process patented by Fischer et al. (2000), which is used in pilot scale manufacture of NOVIHUM™. In this way, smaller quantities of N-modified products from different technical lignins could be chemically characterised and evaluated for phytotoxicity and biomass production in small scale pot tests.

The current study is therefore aimed at the oxidative ammonolysis of the same technical lignins evaluated in 2003, using the ambient pressure process. The investigations were done in the above-mentioned small scale, laboratory plant, and in a pilot scale plant. The effects of the following parameters on the properties of the products were investigated:

- raw material properties
- low pressure vs. high pressure ammoxidation
- lab process vs. pilot process
- using air vs. oxygen as an oxidant
- hydrogen peroxide pre-treatment.

The N-modified products were evaluated in terms of:

- nitrogen content and its binding forms
- carbon: nitrogen ratio (C:N ratio)
- biomass production capability in pot tests. This is discussed separately in chapter 6.

In addition, it was also aimed at the elucidation of the manner in which the various forms of nitrogen are bonded to the lignin macromolecules using Pyrolysis GC/MS, XPS and Cross Polarization Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy (^{15}N CPMAS NMR).

5.2 Materials and methods

5.2.1 Materials

- A bagasse lignocellulosic residue i.e. bagasse partially stripped from sugars for furfural production process (Illovo Sugar Ltd., South Africa).
- A bagasse lignin, trade named Sucrolin, which is produced through solvent extraction of bagasse with weak alkali (Illovo Sugar Ltd., South Africa).
- A calcium lignosulphonate sample (LignoTech, South Africa).
- A Kraft lignin, Indulin AT, obtained from Westvaco Co., USA, was used for comparison.

5.2.2 Oxidative ammonolysis

Oxidative ammonolysis of the technical lignins was carried out in the laboratory and in a pilot plant. According to the patented procedure, the raw material must be suspended in a 5% (V%/V%) ammonium hydroxide solution in a 1:10 solid to liquid ratio. During

the reaction, air/oxygen should be supplied continuously at ambient pressure to oxidise the raw materials. Heat must be supplied at 70°C and the duration of the reaction must be four hours. On completion of the reaction, the products should be allowed to dry at ambient temperature, or in the case of the pilot plant procedure, they can be dried in a drying unit. Except for a difference in the quantity of raw materials taken and equipment, the process conditions used at laboratory and pilot plant scales as shown in table 5-1 were exactly the same.

Table 5-1: Process conditions of the laboratory scale plant and the pilot plant (Ninneman 2002).

Parameter	Laboratory plant	Pilot plant
Ratio of solid to liquid materials	1:10	1:10
Lignite/technical lignin (Solid)	600g	20 000g
Temperature	70°C	70°C
Reaction time	4h	4h
Ammonia concentration	5% or 2.77mol/l	5% or 2.77mol/l
Measurement and control of NH_4OH	Discontinuous*	Continuous
Measurement and control of O_2	Preset**	Continuous

*The ammonium hydroxide concentration was determined every hour and readjusted to the original concentration. ** Incoming air/O_2 into the reactor was regulated on the air pump.

5.2.3 Pre-oxidation of the technical lignins prior to oxidative ammonolysis

Pre-oxidation was carried out by preparing a 1% (V%/V %) hydrogen peroxide solution, and suspending the raw material in the hydrogen peroxide solution in a 1:10 solid to liquid ratio. The suspension was heated at 40°C for four hours. The suspension was maintained by stirring the reactants throughout the reaction. After filtration, the pre-oxidised material was allowed to dry in open air prior to oxidative ammonolysis. The pre-oxidised materials were characterised for carbon and functional group composition as described in chapter 4.

5.2.4 Elemental and functional group characterisation of the products

The elemental and functional group analysis of the products was carried out as described in chapter 4.

The different bonding types of nitrogen, bonded to the lignin macromolecule as **ammonium nitrogen** (NH_4^+-N; easily mineralisable and available to the plant immediately), **amide nitrogen** (NH_2-N; available in the mid-term) and the **strongly organically bonded nitrogen** (Sob-N; available over a long term), were determined according to Wang et al. (1964) and Schiene et al. (1979) as follows:

- The percentage NH_4^+-N was determined by alkaline saponification of the samples (150 mg) with 2g of MgO in 50ml of water for 15 minutes. The ammonium nitrogen released, expressed as the percentage of the total nitrogen, was determined through back titration with 10ml of a 1M HCl solution.
- The percentage NH_2-N was indirectly determined by extracting both the ammonium and amide nitrogen by alkaline saponification of the samples (150 mg) with 15ml of a stronger base i.e. 33% of NaOH solution for 5 minutes. The total amide nitrogen was calculated by subtracting the ammonium nitrogen determined above from this sum.
- The Sob-N was determined by subtracting the sum of the amide nitrogen and ammonium nitrogen from the total nitrogen, as determined by elemental analysis using a CNS analyser (see chapter 4).

5.2.5 Characterisation of the products by analytical techniques

5.2.5.1 Pyrolysis GCMS

Curie-point Pyrolysis GC/MS was performed using a CPP-40 pyrolyser (Fischer/GSG) coupled with a GC 6890 and MSD 5973 (Agilent Technologies). 260 µg of a finely pulverised and freeze dried sample was pyrolysed at 600°C (FecralloyTM) for 10 seconds. The pyrolysate was carried by helium into the inlet (250°C, split 1:20) of the

gas chromatograph. Separation was achieved using a fused silica Optima-5 column (30m, 0.25mm, 25µm), a column flow of 0.9ml/min, an oven programme starting with 50°C (5 min), then 5°C/min to 280°C (2 min) and an auxiliary temperature of 250°C. The mass spectrometer was operated in EI mode at 70 eV, 230°C, and $1.5 \cdot 10^{-5}$ Torr.

5.2.5.2 ^{31}P NMR spectroscopy

Principle: Reagent 1,3,2-dioxaphospholanyl chloride is able to react with OH groups of lignins, carbohydrates, especially those OH groups bearing labile protons (figure 5-1). After derivatisation of the OH groups with the trivalent phosphorous nuclei the material is subject to ^{31}P NMR analysis.

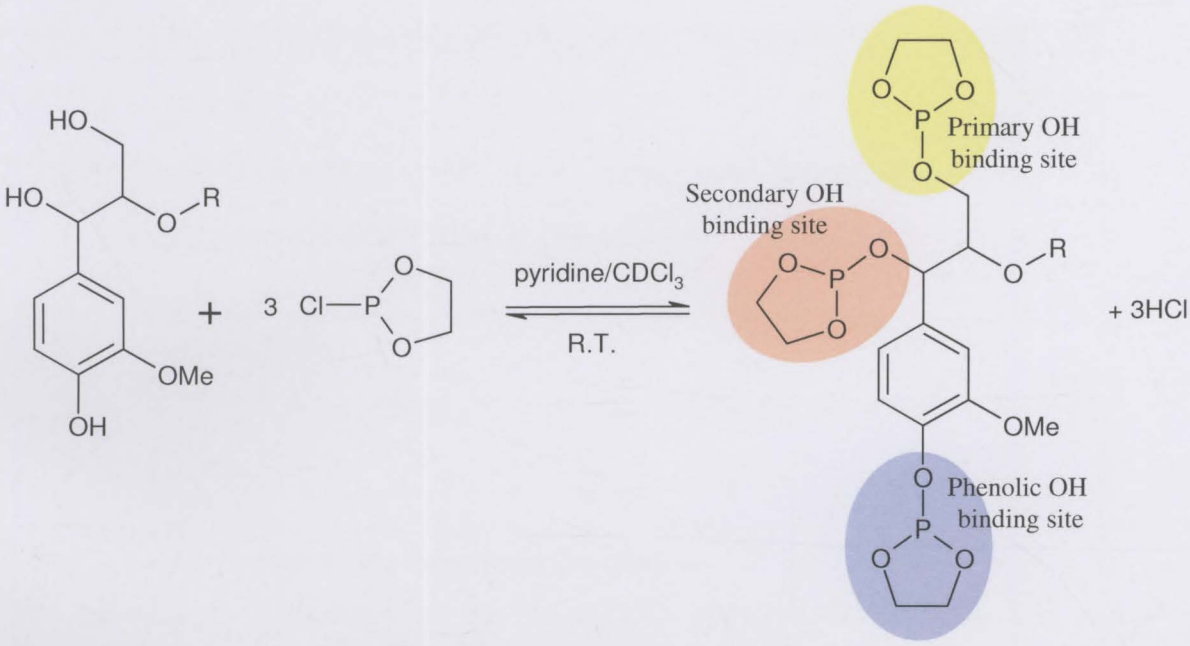


Figure 5-1: Reaction scheme of 1,3,2-dioxaphospholanyl chloride with hydroxyl groups of lignin compounds.

Method:

Solvent Mixture A: pyridine (pre-distilled over NaOH) / deuterated chloroform; ratio 1.6:1.0.

Internal standard: 67.1 mg benzoic acid was dissolved in 1 ml of solvent mixture A

Relaxation reagent: 100 µl of the internal standard was transferred into a 1ml volumetric flask and 2.5 mg of chromium acetylacetonate (Cr(acac)3 was added. The mixture was finally diluted to the mark with the solvent mixture A.

^{31}P NMR spectra were obtained using inverse gated decoupling on a Bruker 400 MHz NMR spectrometer at 162 MHz. The internal deuterium lock was provided by the deuterium atoms present in the deuterated chloroform, used as solvent. The external standard was 85% H_3PO_4. All downfield shifts from H_3PO_4 were considered positive. A sweep width of 2219 Hz was observed and spectra were accumulated with a time delay of 2s between successive pulses. Pulse widths corresponding to a 45° flip angle were used in order to ensure the preservation of uniform bandwidth over the spectral range examined. A line broadening of 4 Hz was used for processing the spectra.

5.2.6 Characterisation of the nitrogen binding forms by instrumental techniques

5.2.6.1 Pyrolysis GCMS

Pyrolysis GCMS of the modified products was carried out as described in section 5.2.5.1.

5.2.6.2 X-ray Photoelectron Spectroscopy (XPS)

XPS spectra were recorded at the Wilhelm-Ostwald-Institute for Physical and Theoretical Chemistry of the University of Leipzig, Germany on a VG ESCALAB 220i XL using Mg Kα-radiation (12 kV, 20 mA) and a pass energy of 10 eV. Correcting of the energy binding scale due to sample charging (1.6 eV) was done by adjusting the C 1s peak of aromatic C-H bonds (EB = 284.8 eV). The energetic resolution of the spectrometer was 0.8 eV (full width of half maximum of the Ag 3d5/2 line at a pass energy of 10 eV). All spectra were fitted with UNIFIT 2006. Fitting, assuming 3 peaks, gave the best fit, lowest residues (deviation from the zero-line below the spectra)

and the obtained full width of half peak maximum (1.7eV) fitted best with literature values.

5.2.6.3 ^{15}N CPMAS spectroscopy-method

For ^{15}N CPMAS studies, Sucrolin was compared to the Lutsatian lignite from Germany. The two raw materials were modified using a 50ml bench scale autoclave system under the reaction conditions described in table 5-3 below. For NMR studies, the raw materials were modified with $^{15}NH_4OH$ to improve sensitivity. ^{15}N solid state NMR measurements were performed on a BRUKER AVANCE 400WB spectrometer (9.4 T) using a CP/MAS probe with 7 mm ZrO_2 rotors. Resonance frequency, sample rotation frequency and recycle delay time were 40.55 MHz, 5 kHz, and 0.5 s respectively. Variable contact time measurements showed a maximum intensity at a contact time of 250 ms which was used for all measurements in natural abundance samples. Decoupling was performed by τ ppm15. Liquid nitro methane d = 0 ppm was used as a reference for ^{15}N chemical shifts which were determined using $^{15}NH_4{}^{15}NO_3$ as external standard. For ^{15}N CPMAS studies, the raw materials were modified with $^{15}NH_4OH$ under the reaction parameters described in table 5-2 below.

Table 5-2: Reaction parameters of the oxidative ammonolysis

Reaction parameter	**Value**
Sample concentration	10% solids (mass%/volume%)
Ammonium hydroxide concentration	5% (V%/V%)
Temperature	70 °C
Reaction time	5 hours
Oxygen pressure	7.5 bars

5.3 Results and discussion

5.3.1 General

According to Schröder (1984), biological degradation of humic materials is hindered at C/N ratios > 25 since micro-organisms need available nitrogen to build up their specific peptides. A significant release of organic bound-nitrogen caused by biodegradation is observed at C/N ratios < 20. Organic matter with a C:N ratio of lower than 20 has a lot to offer in terms of soil conditioning as well as supporting micro organisms in the soil. Since technical lignins have carbon contents ranging from 40 to 60%, the goal of oxidative ammonolysis is, therefore, to incorporate a minimum of 2% nitrogen into the lignin macromolecules to achieve C/N ratios of less than 20. However, for long term application of the products, higher values of nitrogen are required. Technical lignins modified under ambient pressure conditions have been shown to incorporate between 3 and 5% N (Fischer et al. 2002). In humus in the soil, the N content ranges from 1-5%. In this investigation, the N-content values as well as the binding forms of nitrogen for technical lignins and natural humus obtained from literature were used as bench marks for comparison for the N-lignins prepared from the South African technical lignins.

5.3.2 Total nitrogen and C/N ratio

The effects of hydrogen peroxide pre-treatment of the technical lignins and the influence of the reaction conditions (high pressure vs. ambient pressure and air vs. oxygen) in laboratory or pilot scale equipment on total nitrogen and its binding forms, as well as C/N ratio of the products are shown in table 5-3.

Material property effects: In this study at ambient pressure, the N-Sucrolin lignin showed the highest nitrogen content (6.25% N; 9.8 C/N ratio) followed by N-calcium lignosulphonate (3.36%; 12.6 C/N ratio) and the N-lignocellulosic residue (2.64%; 19.50 C/N ratio). The higher reactivity of the Sucrolin had to be expected due to its higher degree of oxidation as shown by its higher content of carboxylic acid groups (see chapter 4). Also by virtue of it being a grass lignin, it had a distinctly lower carbonyl

and methoxyl content than the Kraft lignin, N-Indulin AT (N-content: 3.07%), which is a *Gymnosperm* lignin. In addition, its proportion of the p-hydroxyphenyl units (H), as determined by Curie-Point Pyrolysis GC/MS amounts to about 36%, whereas N-Indulin AT consists almost exclusively of guaiacyl units (95%, G) (see chapter 3). The low response of the bagasse lignocellulosic residue to oxidative ammonolysis can be explained by its considerable cellulosic content. Studies by Meier et al. (1994) on ammoxidation of technical lignins have shown that Kraft lignins were more reactive than lignosulphonates, explaining the higher nitrogen content in N-Indulin AT as compared to the N-calcium lignosulphonate. The C/N ratios of less than 20 indicated that the products were suitable to be used as plant nutrient sources.

The effect of pilot plant vs. lab scale: At pilot scale, the N-Sucrolin and the N-lignocellulosic residue showed lower nitrogen contents as well as higher C/N ratios than at lab scale. This was still within acceptable range for plant nutrient sources and also comparable to other N-lignins as reported in literature (see table 5-3 below).

Table 5-3: Influence of oxygen or air under high or ambient pressure as well as hydrogen peroxide pre-treatment on the elemental composition of the N-modified products. The binding forms of nitrogen were expressed as a percentage of the total nitrogen.

Product	Process conditions			Product characteristics						
				Nitrogen [%] on oven dry basis				Carbon	C/N ratio	total S
	O_2	Air	H_2O_2	Total N	NH_4^+-N	NH_2-N	Sob-N	[%]		[%]
N-Sucrolin	-	**AP (LS)***	-	**6.25**	**38.42**	**13.0**	**48.58**	**61.30**	9.80	**0.21**
	-	**AP (PS)****	-	**4.95**	**29.49**	**9.49**	**61.02**	**58.70**	11.8	**0.54**
Sucrolin †	*HP (LS)**	-	-	***10.05***	*35.80*	*15.10*	*49.10*	*53.51*	*5.34*	
	*HP (LS)**	-	*1%*	***11.80***	*34.87*	*11.70*	*53.44*	*57.07*	*4.84*	*4.84*
	*HP (LS)**	-	*5%*	***12.70***	*34.76*	*11.30*	*53.95*	*56.76*	*4.45*	*4.45*
N-Lignocellulosic residue	-	**AP (LS)***	-	**2.64**	**36.57**	**14.92**	**51.49**	**48.50**	19.50	**0.10**
	-	**AP (PS)****	-	**2.35**	-	-	-	**51.60**	21.95	**0.12**
	AP (LS)	-	-	**3.16**	**28.59**	**11.97**	**59.44**	**47.05**	13.35	**0.12**
	-	**AP (LS)***	**1%**	**3.08**	**26.95**	**14.61**	**58.44**	**47.80**	15.50	**0.12**
	AP (LS)	-	**1%**	**3.59**	**28.13**	**13.37**	**58.50**	**46.50**	12.90	**0.09**
N-Lignocellulosic residue†	*HP (LS)**	-	-	***1.44***	*17.60*	*16.80*	*65.60*	*53.20*	*22.64*	*0.12*
	*HP (LS)**	-	*10%*	***3.72***	-	-	-	*49.20*	*13.20*	*0.09*
N-Lignosulphonate	-	**AP (LS)***	-	**3.36**	**52.70**	**11.90**	**35.4**	**42.00**	12.60	**4.79**
N-Lignosulphonate	*HP (LS)**	-	-	***3.25***	*48.30*	*10.32*	*41.30*	*42.04*	*13.19*	*4.51*
N-Indulin AT	-	**AP (LS)***	-	**3.07**	-	-	-	**60.00**	19.50	**1.76**
N-Indulin AT†	*HP (LS)**	-	-	***3.93***	*62.70*	*14.00*	*23.30*	*61.60*	*15.67*	*1.90*
N-lignins††				**3...5**	10...41	14...21	44...73	60...65	ca. 11	0.6...0.7
NATURAL HUMUS†††				**1...5**	10...25	21...45	ca. 50	41...62	ca. 17	-

Legend AP (LS)*= Ambient pressure, lab scale. **AP (PS) = Ambient pressure, pilot scale. †Values obtained from Tyhoda (2003)* HP (LS) = High pressure. ††Values obtained from Fischer and Schiene (2002). ††† Values obtained from Fischer et al. (2002). The shaded area refers to results of the current investigations.

The effect of oxygen vs. air: Oxidative ammonolysis of the lignocellulosic residue in the presence of oxygen instead of air resulted in a 16.5% increase in N content from 2.64% to 3.16%, as a result of better oxidation of the material that was achieved.

***Pre-treatment with hydrogen peroxide*:** Pre-treatment and oxidative ammonolysis in the presence of air resulted in a 14.3% increase in N content from 2.64% to 3.08%. Pre-treatment and ammoxidation in the presence of oxygen further improved oxidation of the material, and resulted in almost 27% increase in nitrogen (from 3.64% to 3.59%). This phenomenon is consistent with observations by other workers (Meier et al. 1994; Tyhoda 2003).That pre-oxidation had taken place was explained in terms of the change in the functional group composition (the decrease in the methoxy and an increase in the carboxyl group contents) and the decrease in the carbon content of the raw materials as table 5-4 below demonstrates. A decrease in the reducing sugar content after pre-oxidation was also observed.

Table 5-4: The effect of H_2O_2 pre-treatment on the carbon, carboxyl and methoxyl group contents of the South African technical lignins (values given on dry organic matter basis).

Lignin/lignin containing material	Carbon [%]	Carboxyl [%]	Methoxyl [%]	R.S. [%]
*Lignosulphonate-L**	45.20	*3.80*	*9.33*	*10.3*
Lignosulphonate –P**	**42.70**	**8.87**	**7.15**	8.96
Sucrolin –L	*64.20*	*7.61*	*8.30*	*0.86*
Sucrolin –P	**62.90**	**9.92**	**7.88**	0.72
Lignocellulosic residue–L	*51.30*	*2.72*	*4.04*	*0.93*
Lignocellulosic residue-P	**47.80**	**3.91**	**3.81**	0.79

Legend: * L = before pre-treatment. **P = after pre-treatment.

Low pressure vs. high pressure: The results from the 2003 study in table 5-3 indicate that higher nitrogen contents and lower C/N ratios were achieved with the N-Sucrolin and N-Indulin AT through the high pressure process, compared to the ambient pressure process used in the current study. This could be expected due to the fact that, for that

process, oxygen under pressure (7.5 bars) was used, in comparison to air under normal pressure in the current study. Results from other workers also showed that the high pressure process resulted in higher N contents (up to 24%) (Miletzky et al. 1990; Fischer and Schiene 2002). The results obtained with the N-calcium lignosulphonate were similar for both processes while higher nitrogen contents were achieved with the lignocellulosic residue at ambient pressure.

5.3.3 *Different binding forms of nitrogen by wet chemical methods*

In comparison with natural humus, the N-lignins prepared from the South African technical lignins as well as N-lignins described in literature showed a generally higher proportion of NH_4^+-N as table 5-3 demonstrates.

Material properties: The nitrogen binding forms of the N-Sucrolin and the N-lignocellulosic residue were within range of N-lignins as reported in literature (Fischer and Schiene 2002). The proportion of NH_4^+-N in the N-calcium lignosulphonate (52.7%) was on the contrary, much higher than normally observed for N-lignins both under ambient and high pressure conditions, as well as in comparison to values obtained in literature. This was due its high sulphur content as a result of the sulphite pulping process. The sulphur containing groups such sulphonic acid groups introduced as a consequence of sulphite pulping have a particular significance (in lignosulphonates, most of the sulphur is located in sulphonic acid groups) as they direct the reactivity of the lignin macromolecule (Sarkanen and Ludwig 1971). During oxidative ammonolysis, desulphonation takes place only to a small extent. This results in the bonding of NH_4^+ ions corresponding to the anionic groups (Miletzky et al. 1990). Therefore, most of the nitrogen, present as NH_4^+-N, is found bonded to the sulphonic groups of the technical lignin macromolecules. The higher proportion of NH_4^+-N found in the N-calcium lignosulphonates can be detrimental to crops by providing too much nitrogen early in the development of the plant, which can be toxic in the root zone. It could also lead to nitrogen more easily leaching out into ground water.

The effect of pilot plant vs. lab scale: At pilot scale, there was an 8.71 percentage points decrease in NH_4^+-N, from 38.42% at laboratory scale to 29.49% as the table 5-3 illustrates with the N-Sucrolin. The lower the NH_4^+-N in the N-lignins, the better is the product, to avoid nitrogen being easily leached out especially in sandy soils.

Oxygen vs. air: Oxidative ammonolysis in the presence of oxygen resulted in about 8 percentage points decrease in the NH_4^+-N and an increase in about the same margin of the Sob-N, than when air was used. This was due to better oxidation of the raw material during the reaction, which in the end increased the N content of the product.

Pre-treatment with hydrogen peroxide: Pre-treatment of the lignocellulosic residue and oxidative ammonolysis in the presence of air resulted in almost 10% decrease in NH_4^+-N and an increase in Sob-N by almost the same margin (9.96%). When oxygen was used the decrease was 8.44 percentage points, which was marginally different than when air was used.

Low pressure vs. high pressure: During the lab-scale oxidative ammonolysis, the milder conditions of the ambient pressure process generally resulted in an increase in the proportion of the immediately available nitrogen, NH_4^+-N. Figure 5-2 illustrates this phenomenon with N-Sucrolin. High pressure and high temperature causes the formation of more active sites and thereby creating more possibilities for nitrogen incorporation, however, higher temperatures can cause decarboxylation (T>=200°C) This also increases the probability of certain radical reactions – recombination and formation of more stable compounds. They can also result in the conversion of carboxyl groups into amides which cannot bind NH_4^+ anymore.

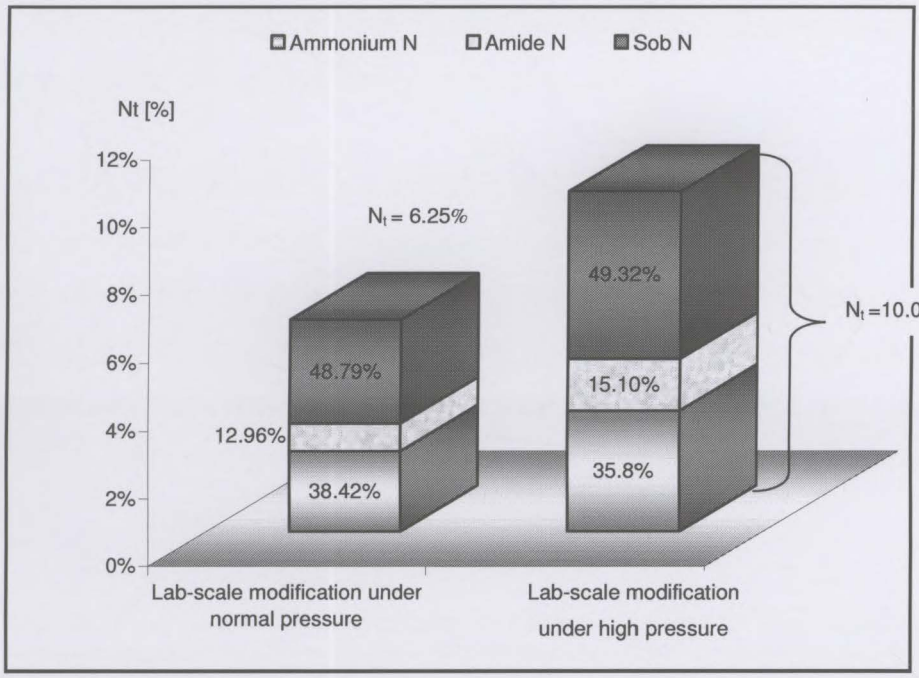


Figure 5-2: Comparison between the effect of the high pressure and low pressure oxidative ammonolysis processes on N-binding forms.

5.3.4 Characterisation of the products by instrumental techniques

5.3.4.1 Pyrolysis GCMS

Altogether 90% of the total peak areas obtained by TIC integration could unambiguously be assigned to specific organic compounds. 75 to 100 of those compounds were assigned in each raw material. Most of them were in turn grouped according to their origin i.e. a group representing carbohydrate pyrolysis products and the different groups formed by thermal degradation of the (substituted) lignin building units (p-hydroxyphenyl (P), guaiacyl (G) and syringyl (S) units) (table 3-1). However, when calculating their pyrolytic lignin portion it has to be taken into account that the same amount of any two compounds in the pyrolysates may give different results in the MS detector. To get correct values, the response factors of all individual compounds contained in pyrolysates have to be known. This is rather difficult with pyrolysis-GC/MS, since a couple of unknown products are usually formed under pyrolytic conditions. Hence, the calculated pyrolytic lignin and carbohydrate contents should be considered as approximate values having a possible error range of about 5% according

to response measurements done on a number of low-molecular substances obtained during thermal lignin degradation (Scholz et al. in press).

At the beginning of the pyrolysis, the gas peak appeared after the dead-time (time from start to the appearance of the first eluating compound) up to a retention time of 2.96min. Since this peak consisted of several co-eluating compounds (CO_2, CO, H_2O, SO_2, CH_4, NH_3, HCOOH, HCHO, CH_3COOH, CH_3OH etc.), its broad signal was not resolved and no compound assignments were made. The total area was handled as a "gas peak" referring to all those compounds which easily degraded under the specific pyrolytic conditions.

Ammoxidation using the ambient pressure technology seems to be a mild oxidation causing smaller changes of the molecular structure of the technical lignins than is the case with the high pressure technology. In hydrogen peroxide pre-activation, the relatively strong oxidative conditions result in the loss of some portions of the "weakly bound organic matter". The influence of oxidation during oxidative ammonolysis after activation was rather limited. The carbohydrate content decreased as expected during both reactions i.e. during pre-activation with hydrogen peroxide (in agreement with wet chemical methods, see table 5-2) as well as during ammoxidation. In essence, oxidation resulted in the loss of organic matter (formation of CO_2 which was released from the reaction mixture) and the formation of low-molecular weight degradation products which were more easily converted to compounds contributing to the "gas peak".

The total lignin content increased as a result of the decrease in the proportion of other compounds. A small amount of lignin could also have become resistant to degradation due to re-arrangement of labile bonds and formation of more stable compounds.

Table 5-5: Structural characteristics of the technical lignins before and after N-modification.

Technical/N-lignin*	Sum of explained peaks starting from 2.96min (%)	Sum of explained peaks (%)	Gas peak area (%)	Lignin (%)				Carbohydrates (%)	N-compounds (%)
				Total	P	G	S		
Sucrolin	90.7	73.9	18.5	86.0	37.1	44.0	18.9	3.6	
N-Sucrolin	89.8	67.2	25.1	86.9	38.2	28.8	32.9	1.8	2.1
P-Sucrolin	90.2	75.6	16.2	87.1	35.2	38.3	26.5	2.4	
N-P-Sucrolin	89.1	74.3	16.6	87.3	36.5	31.6	31.8	0.5	0.9
Calcium lignosulphonate	94.9	18.8	80.2	56.9	9.3	70.0	20.7	36.8	
N-Calcium lignosulphonate	97.9	32.0	67.4	63.3	8.2	64.2	27.6	22.9	10.2
N-P-Calcium lignosulphonate	92.3	20.7	77.6	59.9	13.3	61.2	25.4	28.4	
Lignocellulosic residue	74.4	54.8	26.4	43.6	36.3	40.5	23.2	29.0	
N-Lignocellulosic residue	75.6	54.5	28.0	47.0	45.9	42.3	11.8	20.8	6.1
P-Lignocellulosic residue	79.5	55.2	30.6	54.1	50.1	26.8	23.1	23.4	
N-P-Lignocellulosic residue	87.7	62.5	28.7	52.7	40.7	39.4	19.9	23.3	6.8

*N refers to N-modified technical lignin and P refers to pre-activated technical lignin.

The increase of P units from the two raw materials i.e. Sucrolin and P-Sucrolin to the corresponding N-modified products i.e. N-Sucrolin and N-P-Sucrolin can be clearly seen (see table 5-5). This was expected as a result of the degradation of G and S units (cleavage of methoxyl groups). The G units were also expected to decrease corresponding to the increase of P units as explained for Sucrolin. However, the G content could also have increased if the degradation of S units was preferred than the degradation of G and P units. The increase in S units can only be explained by the degradation of P units, i.e. when certain portions of lignin decrease during oxidation/ammoxidation, others increase.

5.3.4.2 ^{31}P NMR spectroscopy

Sucrolin consisted of all three types of lignin units (syringyl, guaiacyl and p-hydroxyphenyl units which are characteristic of grass lignins. In contrast, the Indulin AT, a mixed softwood lignin mainly contained guaiacyl units which resonate at about 131ppm after derivatisation with 1,3,2-dioxaphospholanyl chloride. Primary alcohol groups resonated at about 134ppm. Sucrolin was also more oxidized than Indulin AT as shown by its high proportion carboxyl groups (see table 4-2, section 4.4.2). This might also explain the broadened signal in the range of 128.5ppm where the phosphorous atom attached to the COOH group of benzoic acid, the internal standard, resonated (figure 5-3). Furthermore, Sucrolin had about one primary alcohol per phenolic unit and a rather low content of β-O-4 structures which is in agreement with results from literature (Argyropoulos 1994). After modification, the content of primary alcohol groups decreased (*cf.* peak at δ 134.27 ppm) which could indicate that some condensation reactions or the cleavage of a certain proportion of side chains had taken place. In addition, the content of syringyl, guaiacyl and p-hydroxyphenyl units increased. This could be seen from the ratio of benzoic acid (internal standard) / Σ of S, G, and P units which increased from about 1:3.7 to about 1:6 which could be due to the cleavage of originally esterified phenolic groups caused by ammoxidation or cleavage of aliphatic side chains.

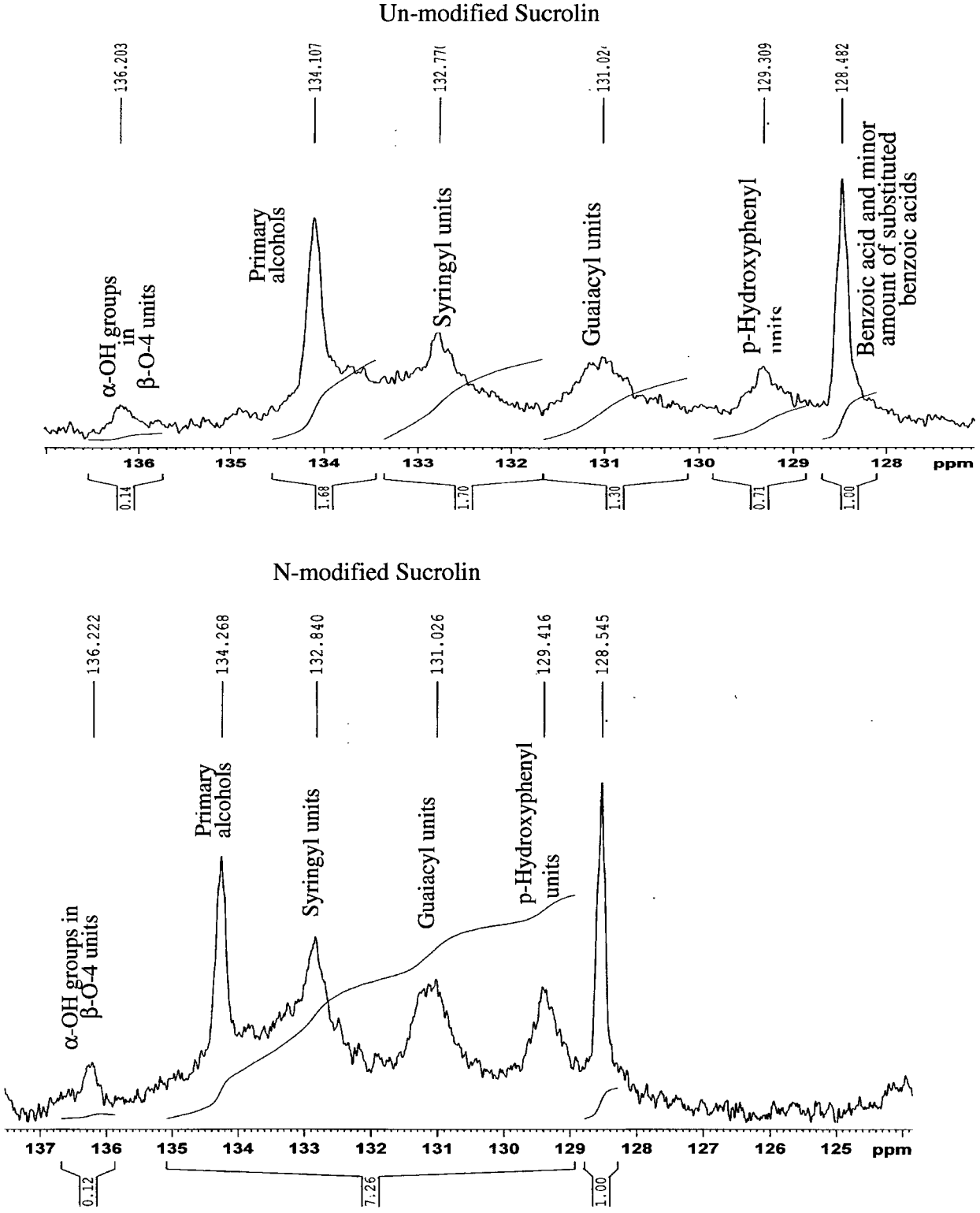


Figure 5-3: ^{31}P NMR of spectra of Sucrolin lignin prior to (top) and after oxidative ammonolysis (bottom).

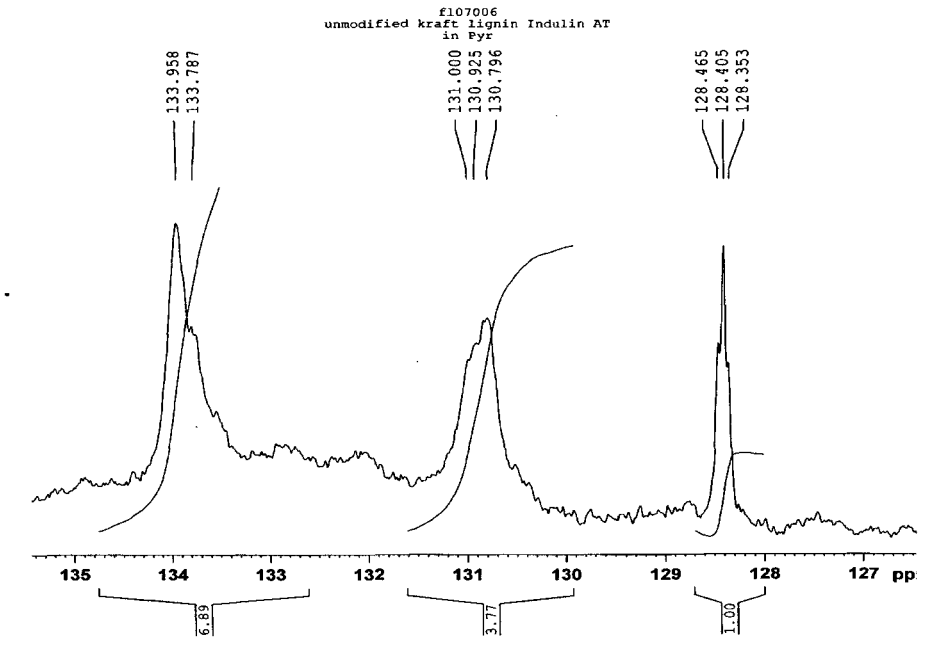


Figure 5-4: ^{31}P NMR spectrum of Kraft lignin Indulin AT prior to oxidative ammonolysis.

5.3.5 *Analysis of different binding forms of nitrogen by instrumental techniques*

5.3.5.1 Pyrolysis GCMS

Analytical pyrolysis of N-modified Sucrolin prepared under high pressure yielded the nitrogen containing thermal degradation products shown in figure 5-5 below. The structures of the N-containing products are also given in figure 5-6. As figure 5-5 illustrates, for the N-Sucrolin prepared under high pressure conditions, a couple of N-containing compounds could be assigned to individual structures. Apart from formamide, acetamide, urea and N-acetylurea (the latter probably being formed in gas phase from urea and acetyl radicals), N heterocyclic compounds such as pyrrole and pyrazine derivatives could also be detected. Furthermore, typical N-containing lignin pyrolysis products like the amides or nitriles of 4-Hydroxybenzoic acid, 4-Hydroxy-3-methoxybenzoic acid (vanillic acid) and 4-Hydroxy-3,5-dimethoxybenzoic acid (syringic acid) were formed. Contrary to the N-Sucrolin prepared under high pressure, in N-Sucrolin prepared under the patented ambient pressure process, fewer of the N-containing compounds shown in figure 5-5 below could be found.

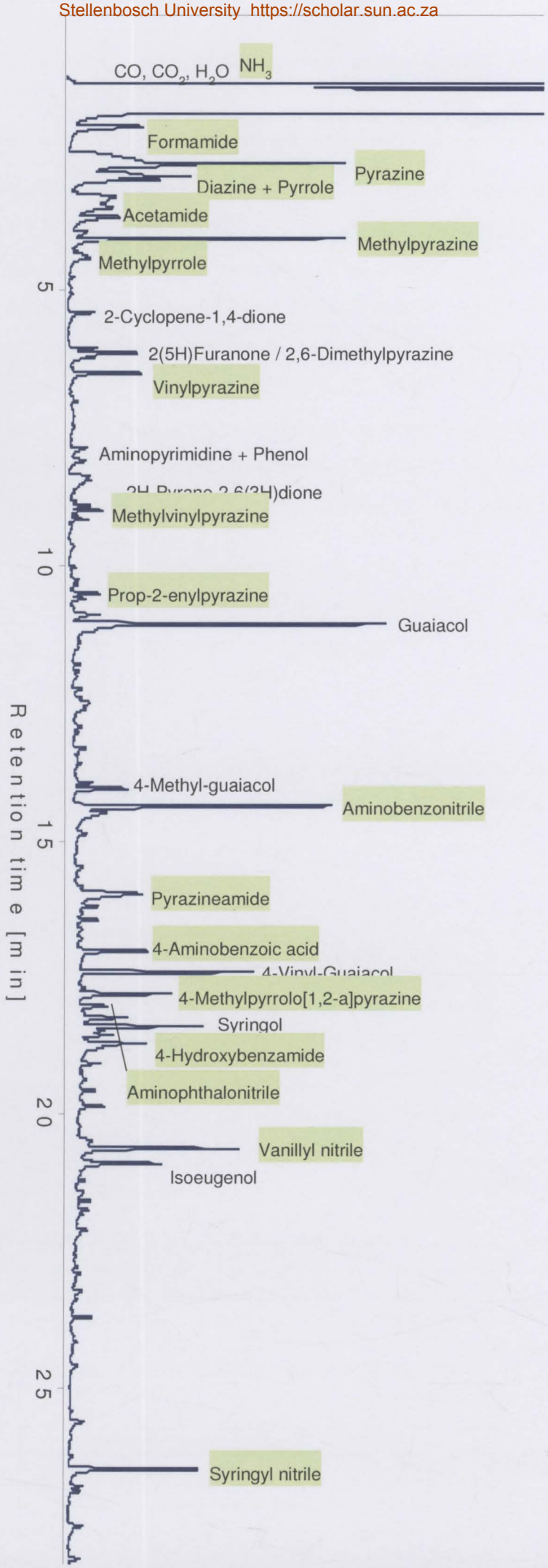


Figure 5-5: Total ion current chromatogram of the Curie-point pyrolysis GC/MS thermal degradation data of nitrogen containing products of N-Sucrolin obtained from the high-pressure technology.

In addition, the proportion of N-containing pyrolysis products peak areas were distinctly lower compared to the N-Sucrolin prepared under the high pressure process. However, formamide, acetamide, urea and N-acetylurea as well as several methylpyrroles were found. Benzoxazole and 3H-Benzooxazol-2-one derivatives were also formed probably through the reaction of acetic amide with the corresponding resorcin derivatives. In a quite similar way, Benzo[1,3]dioxol-2-one was formed probably from carbon dioxide and ortho-Hydroquinone. The formation of Isoindole-1,3-dione clearly started from o-Phthalic acid which underwent a condensation reaction with free ammonia which was released from the ammonia groups of the N-modified sample. This represented about 30% of the total nitrogen content of the sample.

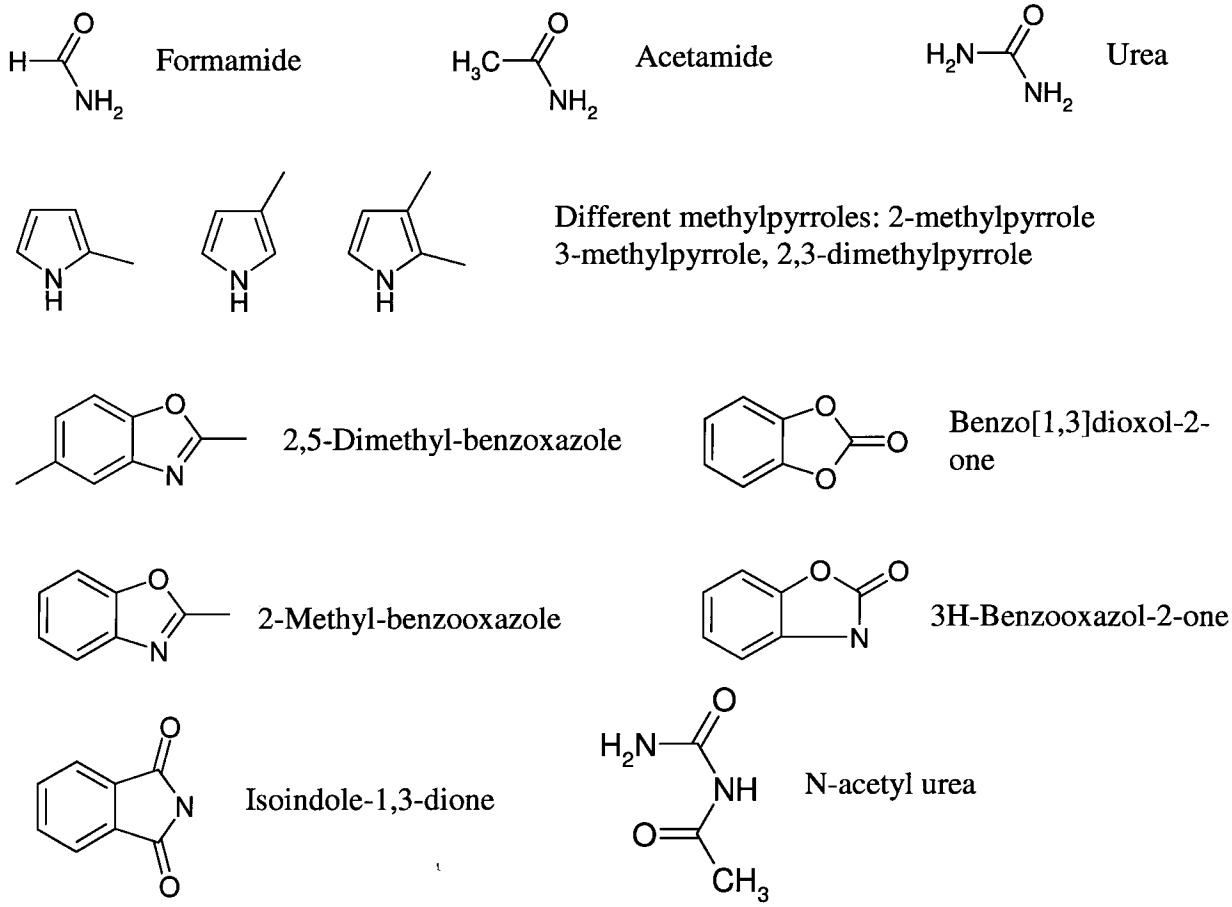
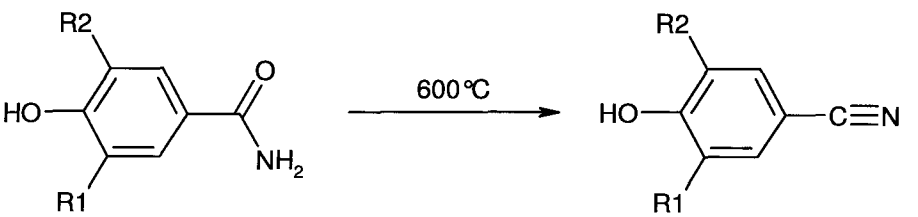


Figure 5-6: N-containing pyrolysis products found in the pyrograms.

In order to be able to assign all N containing lignin compounds originating from pyrolysis products, the same procedure described in chapter 3 was followed for 4-Hydroxy-3,5-dimethoxyphenylamide and nitrile. Literature concerning the occurrence of 4-Hydroxyphenyl and 4-Hydroxy-3-methoxyphenylamides in N-modified organic matter and their transformation into the corresponding nitriles upon pyrolysis is very limited. In addition, the mass spectra of the 4-Hydroxy-3,5-dimethoxyphenylamide and nitrile have not yet been reported in the literature. Therefore, in order to obtain the mass spectra and to study the transformation of the amides into the corresponding nitriles under pyrolytic conditions (figure 5-7), 4-Hydroxy-3-methoxyphenylamide and 4-Hydroxy-3,5-dimethoxyphenylamide were synthesized from vanillic acid and syringic acid, respectively. According to the Schotten-Baumann reaction, the respective carboxylic acid was reacted with thionyl chloride to give the corresponding acid chloride which was subsequently transferred into the amide by reacting with 30% aqueous ammonia. In this way, the behaviour of the homologous series 4-Hydroxybenzoic amide, 4-Hydroxy-3-methoxybenzoic amide, and 4-Hydroxy-3,5-dimethoxybenzoic amide shown in figure 5-8 which are formed under pyrolytic conditions could be studied. The mass spectra of the corresponding nitriles are shown in figure 5-9 below.



R1, R2=H
R1=OCH3, R2=H
R1, R2=OCH3

Figure 5-7: The transformation of the amide into the corresponding nitriles under pyrolytic conditions.

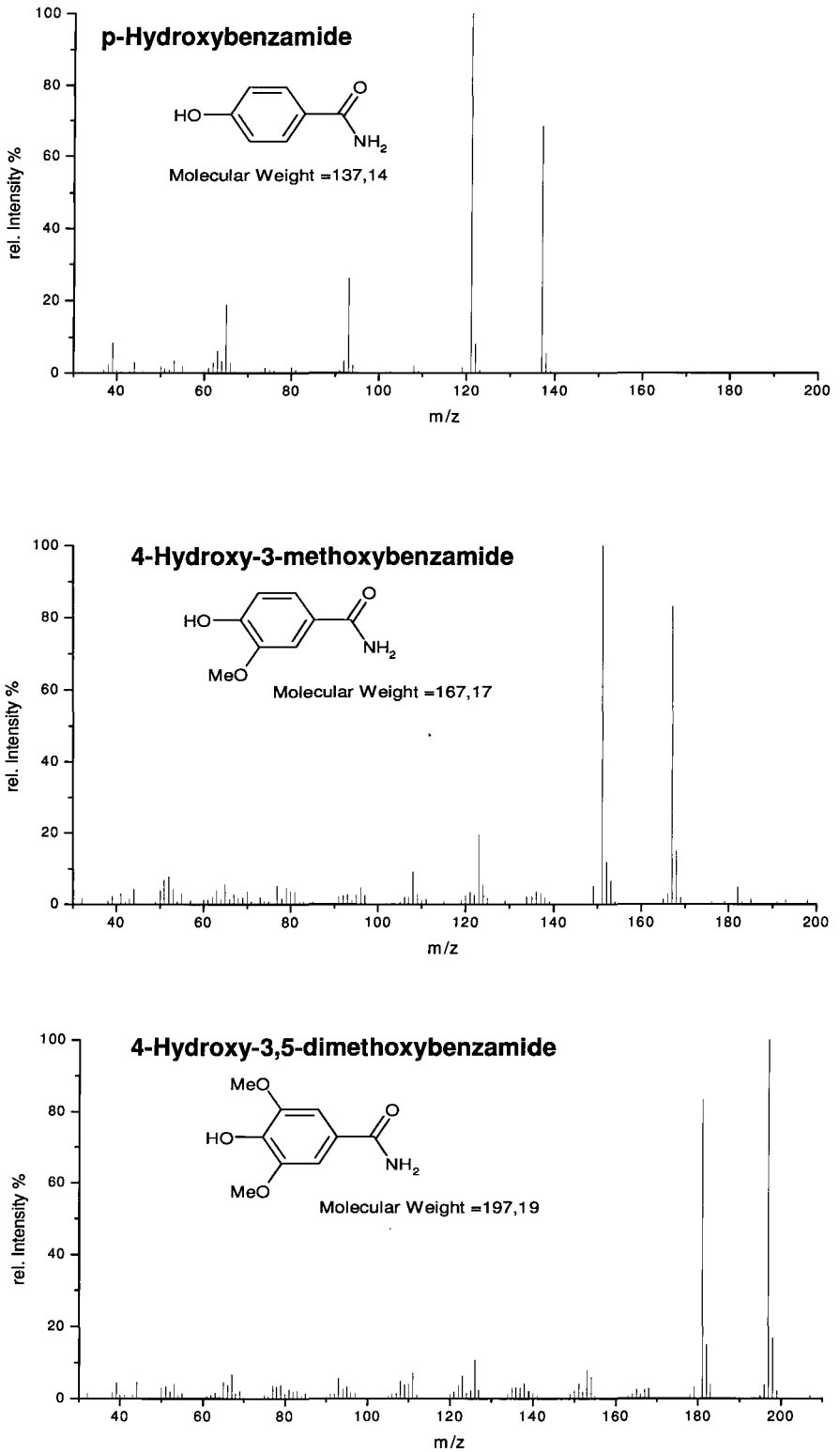


Figure 5-8: Mass spectra of homologous series of benzoic acid amides

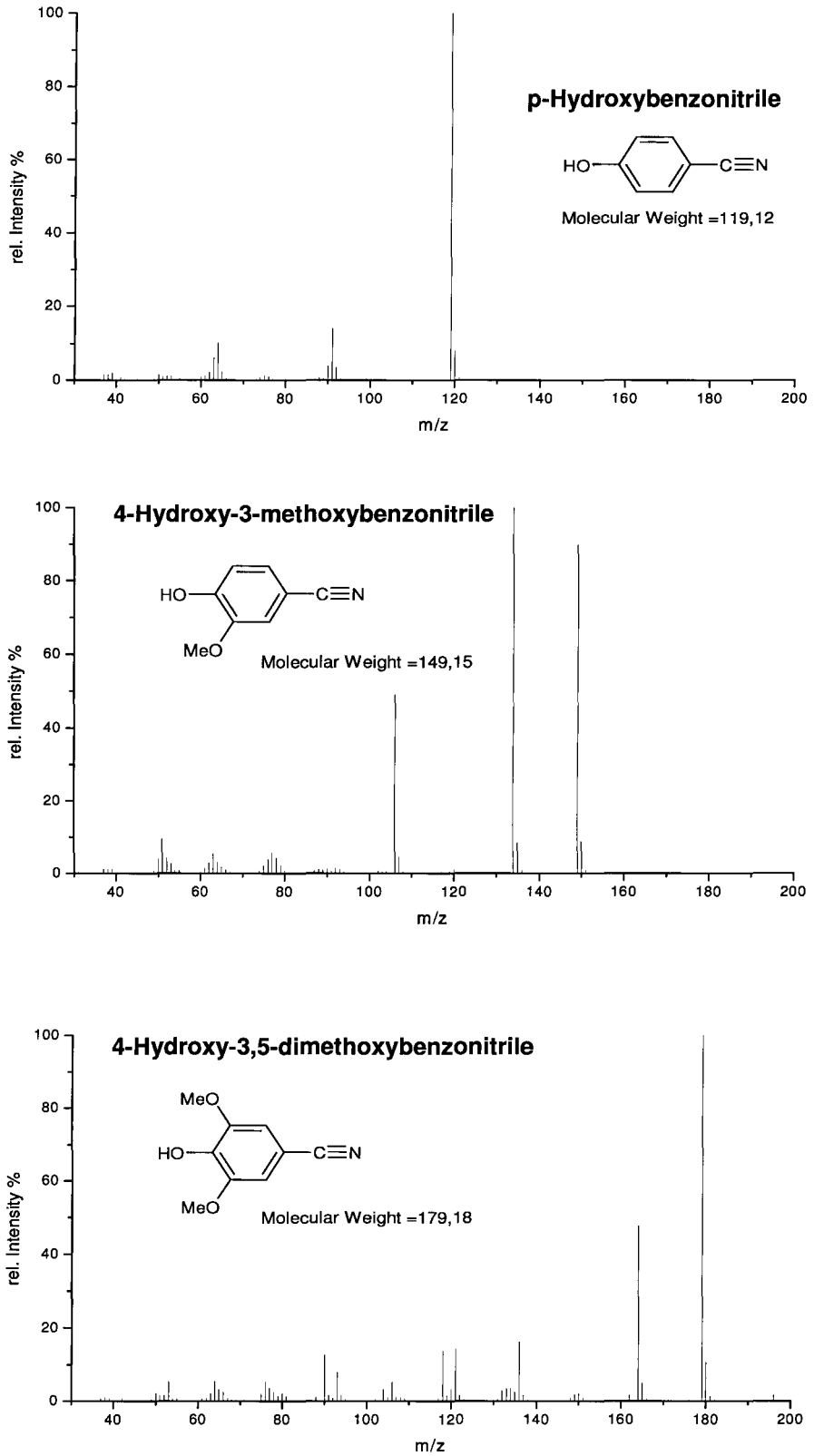


Figure 5-9: Corresponding nitriles of the benzoic acid amides formed under pyrolytic conditions.

5.3.5.2 X-ray Photoelectron Spectroscopy

Figure 5-10 shows similarities in the photoelectron spectra of N-Sucrolin and N-modified Miocene lignite. As figures 5-11 and 5-12 illustrate, the distribution of the three main N-binding forms of nitrogen i.e. ammonia, amide/pyrrole and amine/pyridine in the modified lignite and N-Sucrolin was similar. It was not possible to distinguish between amide and pyrrole nitrogen due to the very similar N-binding energy of these two N-binding forms. For the same reason, it was also not possible to distinguish between amine and pyridine. However, due to the dominance of amide derivatives in the Pyrolysis GC/MS spectra, as well as the results from ^{15}N CPMAS NMR studies, where almost no heterocyclic N could be found, it could be concluded that the highest portion of N in ammoxidized Sucrolin was bound to amide-type aliphatic and aromatic compounds. The amide/pyrrole proportion was higher in N-Sucrolin (60.1%) than in N-modified lignite (53.5%). From the O 1s spectra (figures 5-13 and 5-14) it could be observed that the N-modified lignite contained more C=O groups compared to N-Sucrolin.

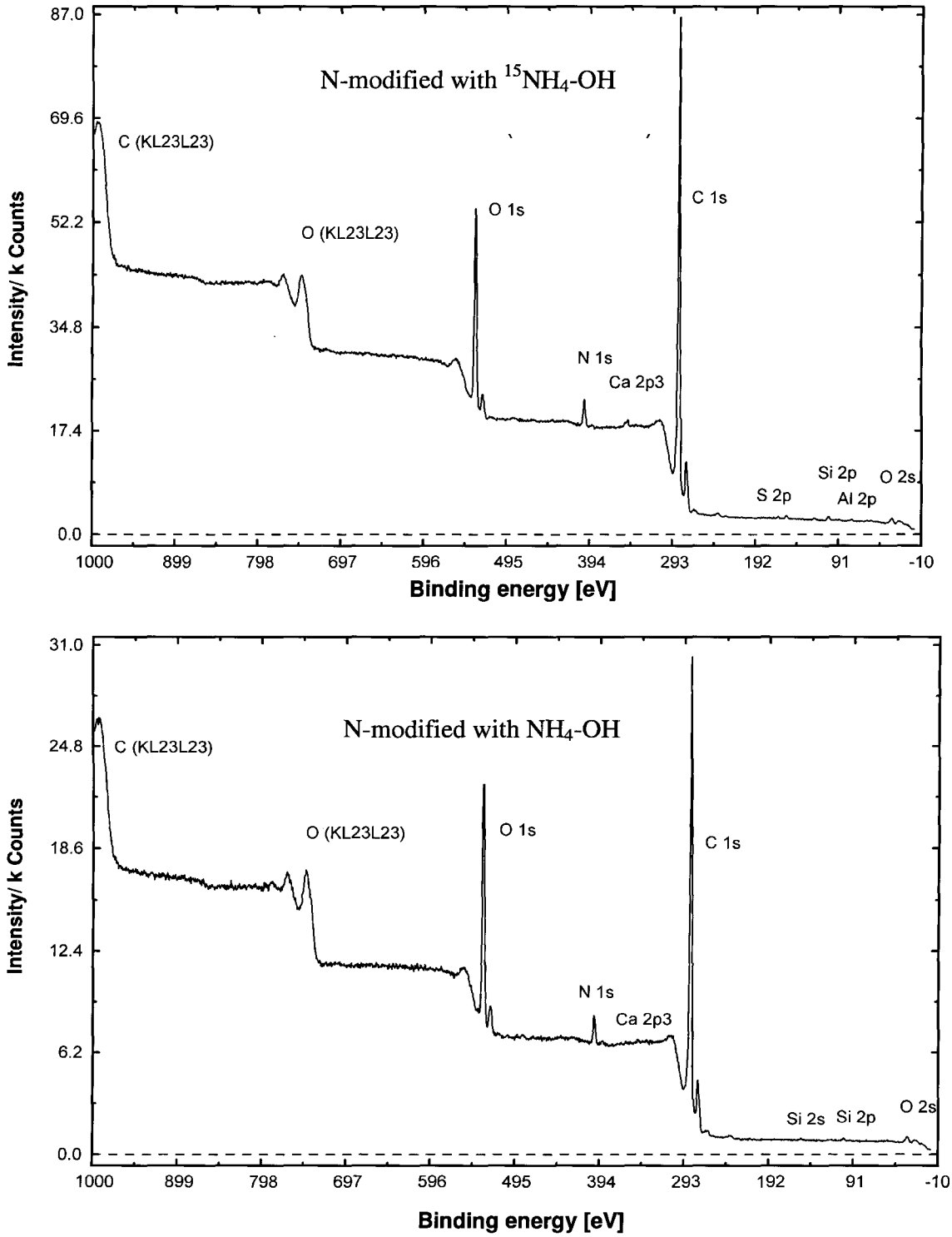


Figure 5-10: Photoelectron overview spectra of ammoxidized Miocene lignite (NOVIHUM™) from the lignite district, Germany (top) and of ammoxidized South African sugar cane bagasse lignin Sucrolin (bottom).

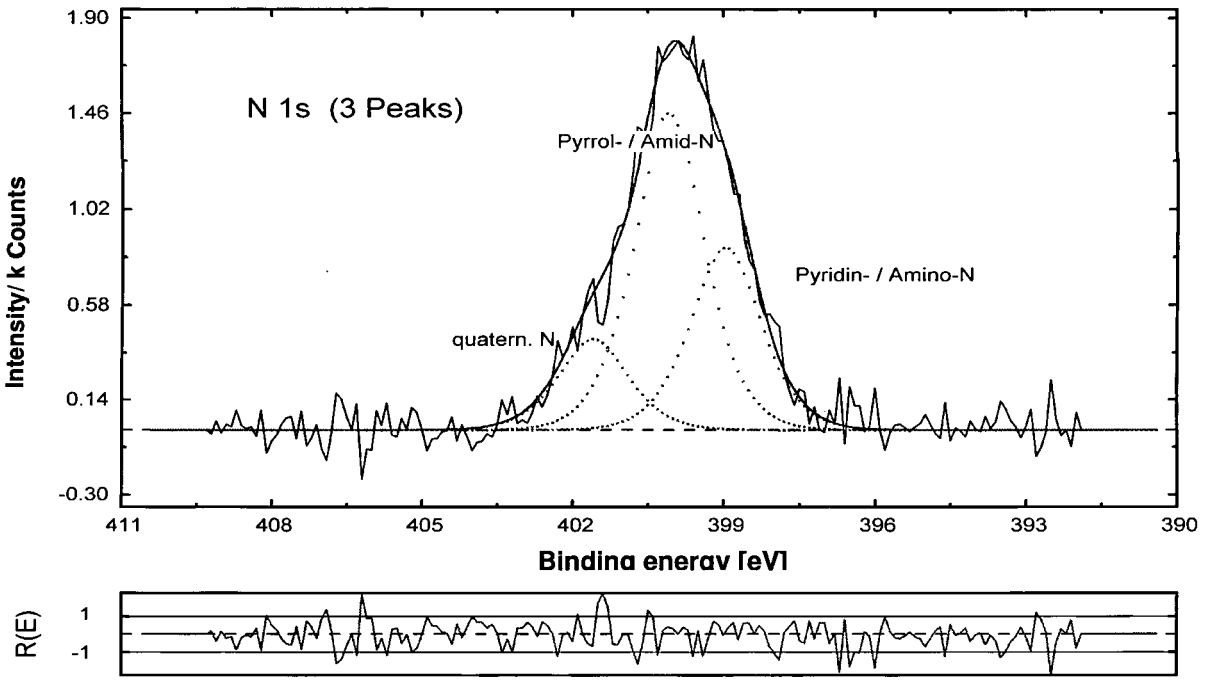


Figure 5-11: N 1s photoelectron spectrum of ammoxidized lignite (E_B = 399.0 eV [31%]; 400.1 eV [53.5%]; 401.6 eV [15.5%]; HWB = 1.7 eV).

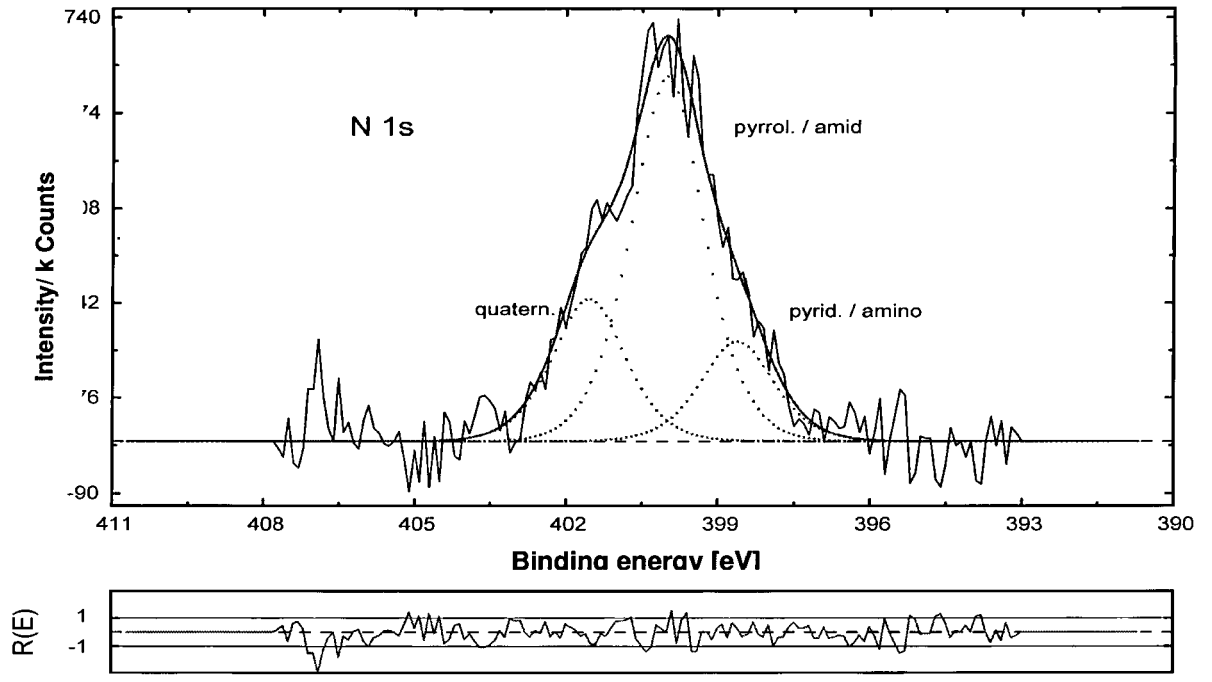


Figure 5-12: N 1s photoelectron spectrum of ammoxidized Sucrolin (E_B = 398.6 eV [16.4%]; 400.0 eV [60.1%]; 401.5 eV [23.5%]; HWB = 1.76 eV).

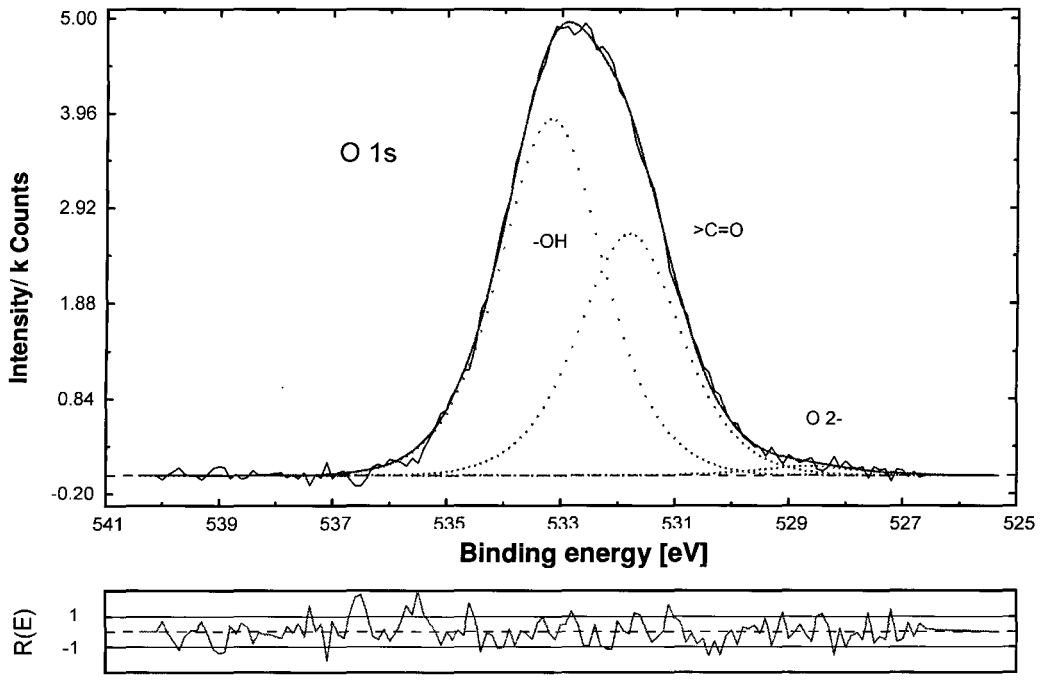


Figure 5-13: O 1s photoelectron spectrum of ammoxidized lignite (E_B = 528.9 eV [0.3%]; 531.7 eV [45%]; 533.1 eV [54%]; HWB = 2.4 eV).

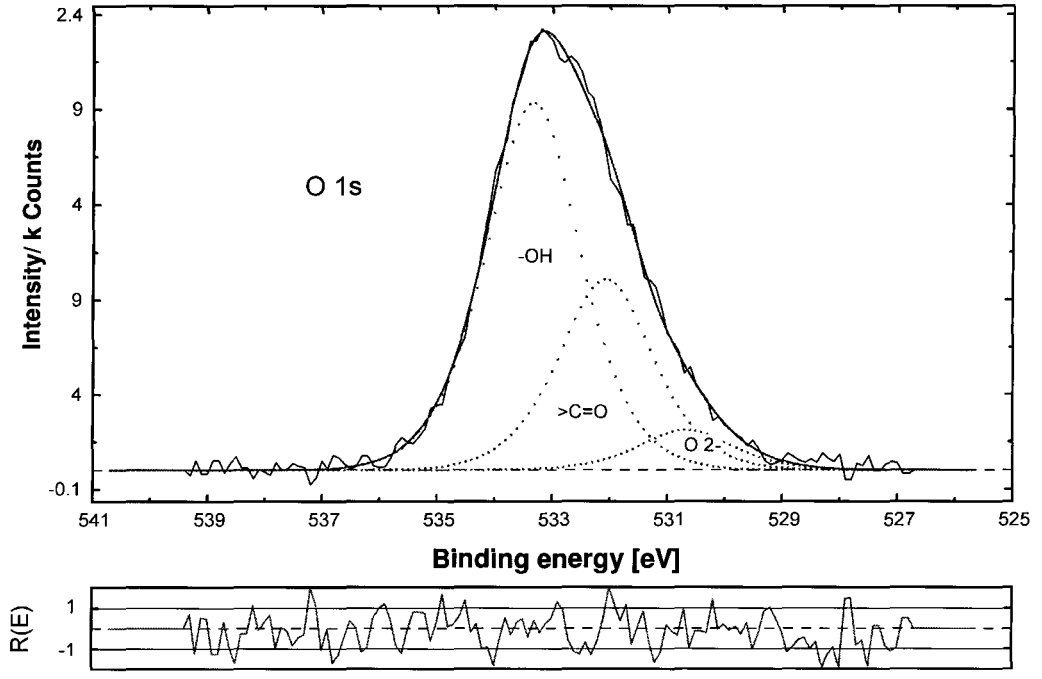


Figure 5-14: O 1s photoelectron spectrum of ammoxidized Sucrolin (E_B = 530.7 eV [6.7%]; 532.1 eV [32%]; 533.3 eV [61.3%]; HWB = 2.01 eV).

5.3.5.3 ^{15}N CPMAS NMR spectroscopy

The results of ^{15}N CPMAS NMR spectroscopy showed a similar distribution of N-binding forms as the XPS measurements. As expected, more groups of N-binding forms were found with ^{15}N CPMAS NMR spectroscopy than could be found with XPS. In fact, surface analyses may be unreliable due to surface contamination. Hence a complimentary technique such as NMR should be used. Chemical forms often give distinct results in the XPS, which are analogous to the chemical shift in NMR (Lambert et al. 1999). The unfavourable gyromagnetic ratio as well as the low natural occurrence of the ^{15}N nucleus does not favour NMR measurements of nitrogen containing compounds (it is the cause low detection sensitivity). Hence, the sensitivity was increased by carrying out oxidative ammonolysis with ^{15}N labelled ammonium hydroxide to improving detection sensitivity. In addition, contact time (time for polarisation transfer) must be optimised for each type of sample. However, the same experimental setup as developed for N-modified lignite (NOVIHUM™) was used for acquiring the ^{15}N CPMAS NMR spectra of ammoxidized Sucrolin (see figure 5-15). Preferably, contact time should be 250 ms.

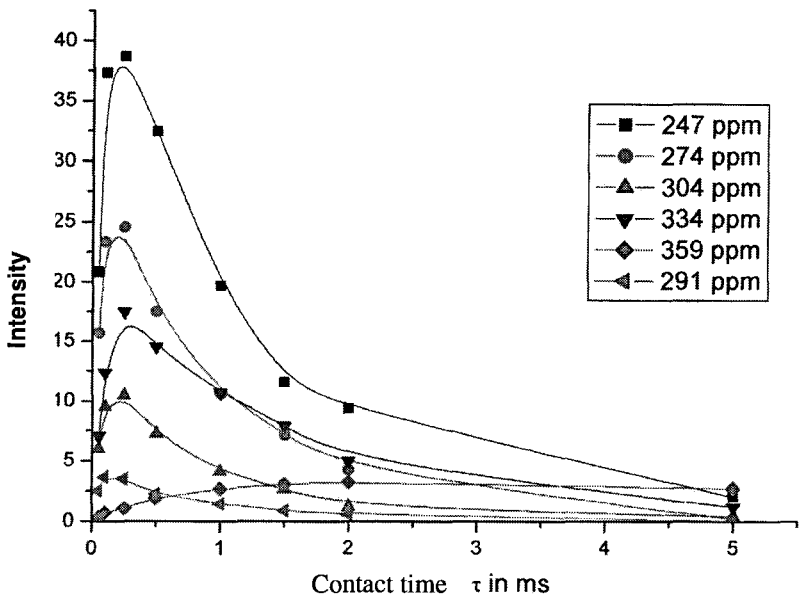


Figure 5-15: Contact time optimisation curve.

In essence, the patterns of results between XPS and ^{15}N CPMAS for N-modified lignite and N-Sucrolin were found to be similar i.e. amides or similar structures (e.g. polyamides) were dominant compared to amines, urea and ammonia groups (easily-bound nitrogen) (see figures 5-16 and 5-17). A rather low content of N-heterocyclic compounds was found in accordance with Pyrolysis-GC/MS where it was difficult to detect and assign N-containing pyrolysis products. This was due to low sensitivity caused by small and broad signals which were found. As such, mainly amide and urea derivatives were found. As already described above, mainly pyrrole derivatives, formamide, acetamide, urea, N-acetylurea, benzoxazole, 3H-benzooxazol-2-one derivatives, benzo[1,3]dioxol-2-one and isoindole-1,3-dione were formed.

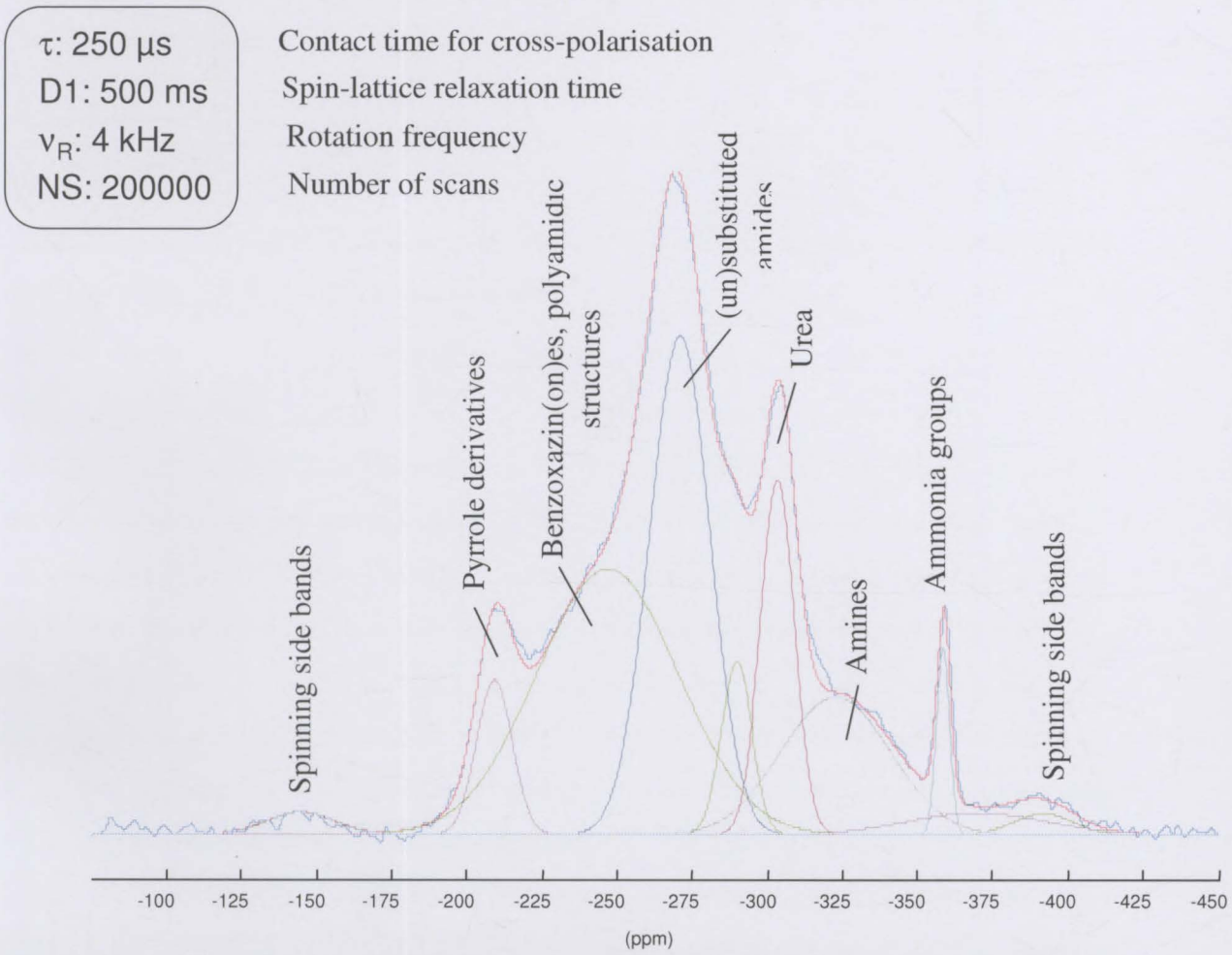


Figure 5-16: ^{15}N CPMAS NMR spectrum of N-modified Miocene lignite from the lignite district (Germany) using $^{15}NH_4OH$ for ammoxidation (in order to increase the sensitivity).

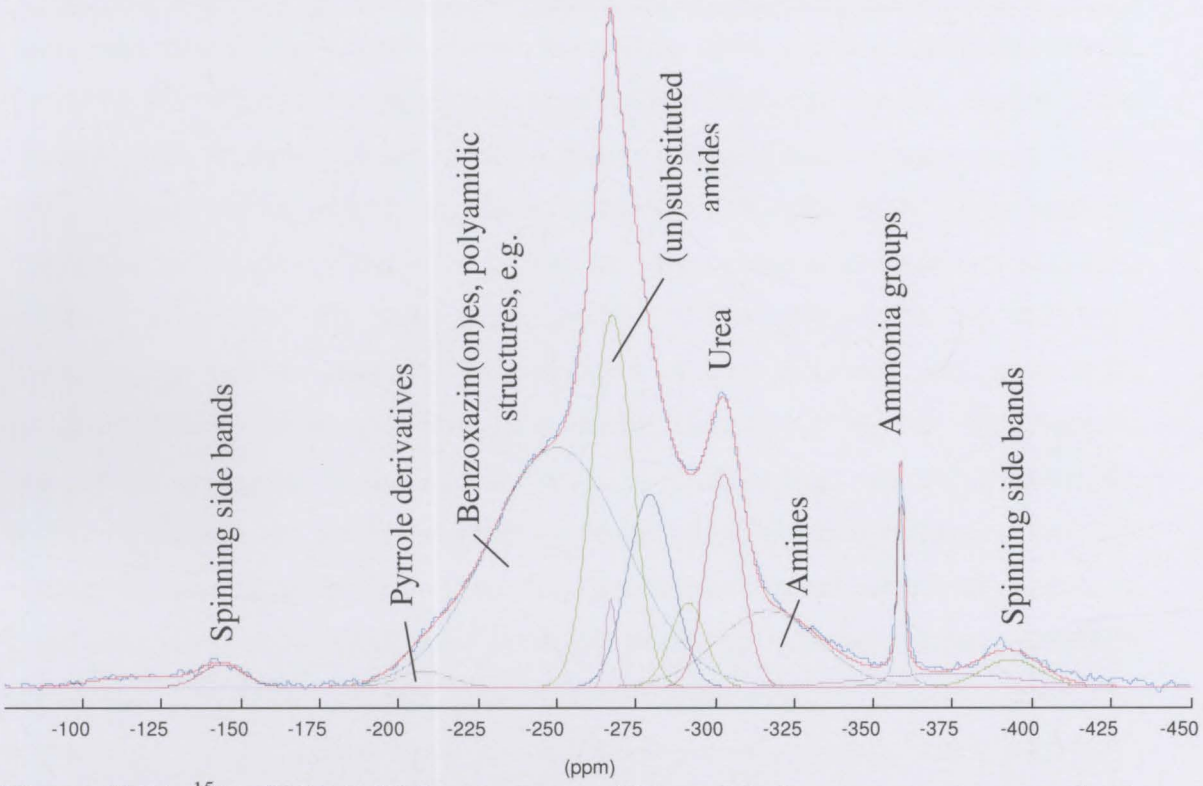


Figure 5-17: ^{15}N CPMAS NMR spectrum of N-modified sugar cane bagasse lignin Sucrolin using $^{15}NH_4OH$ for ammoxidation.

5.4 Conclusion

The three raw materials investigated were distinctly different and hence responded differently to oxidative ammonolysis. The Sucrolin lignin compared with calcium lignosulphonate and the lignocellulosic residue, being a grass lignin derived from steam explosion, had a relatively low non-lignin content resulting from ash and carbohydrates. The best response in terms of nitrogen incorporation was therefore obtained with this raw material. The lignocellulosic residue, due to its considerably higher content of cellulosic material had lower N content and the calcium lignosulphonate was in the mid range of the two. In comparison with Indulin AT, the Sucrolin responded better i.e. a higher nitrogen content was obtained. In general, all the materials had lower C/N ratios (< 20), which proved that they had adequate nitrogen to be used as soil nutrient improving materials.

More also reported in literature, lower N–contents were expected under the normal pressure process than under the high pressure process. Although lower N contents were obtained under ambient pressure conditions, they were within the range acceptable for soil fertilising materials, which was shown by the low C/N ratios (>20). Under ambient conditions, the Sucrolin yielded the highest nitrogen content than the other three raw materials as it also did under high pressure. The results obtained with the lignocellulosic residue, although lower in terms of total N content, due to its high cellulose content, were within the range normally found with N-lignins. This opens a new area of application of this material which is found in huge quantities around the world. In addition, the results show that the bagasse lignocellulosic residue can be used without the removal of the lignin (Sucrolin), which has to be extracted with chemicals. It had a high content of carbohydrate (as shown in chapter 3), however, the results have shown that a huge bulk of this component was glucose 87.2% and the xylose; the component of major interest as far as phytotoxicity was concerned was a negligible 0.65% (see chapter 4). It was shown that oxygen feeding to the reaction mixture instead of air as well as hydrogen peroxide pre-treatment increased the nitrogen content which was in agreement with previous studies.

In comparison with natural humus, the N-lignins prepared from the South African technical lignins showed a generally higher proportion of NH_4^+-N. This is generally the case with N-lignins reported in literature. The N-Sucrolin and the N-lignocellulosic residue were, however, still within the (10...41%) of N-lignins as reported in literature. Contrary to this, the proportion of NH_4^+-N in the N-calcium lignosulphonate (52.7%) was much higher than (10...41%) observed for N-lignins manufactured both under ambient and high pressure conditions, as well as when compared to values from literature. This was mainly due to the sulphite pulping process with which it is isolated from wood.

Under the ambient pressure process, oxidation seemed rather mild causing only smaller changes in the molecular structure of the technical lignins as compared to the high pressure. However, with pre-oxidation with peroxide the relatively strong oxidative

condition result in removal of the "weakly bound organic matter" from the technical lignins. ^{31}P NMR spectroscopy, in agreement with wet chemical methods showed that the Sucrolin was more oxidized than Indulin AT as shown by its high proportion of carboxyl groups. The primary alcohol group content of the Sucrolin decreased after modification, indicating that some condensation reactions or the cleavage of a certain proportion of side chains had taken place.

A combination of various analytical techniques provided insight into the nature of N-binding forms of N containing compounds in N-lignins prepared under ambient pressure. While it was difficult to assign peaks for N containing compounds in general with pyrolysis GCMS due to low sensitivity as shown by small and broad signals, more information could be obtained from ^{15}N CPMAS NMR spectroscopy. The N-Sucrolin showed a similar distribution of the N-binding forms as the N-modified lignite. The study, complementary to the wet chemical analysis, has given a full picture of the N-Sucrolin in comparison with N-modified lignite (NOVIHUM™, the reference product) and provided a benchmark for comparison with other N-lignins as far as the distribution of nitrogen in the N-lignins are concerned. The three analytical methods showed that amides seem to be the main N-containing fractions in N-lignins as well as N-lignites prepared under ambient pressure.

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6.0 Pot performance trials of N-modified technical lignins with yellow mustard, *Sinapis alba*

Abstract

Biomass production of mustard seed, *Sinapis alba,* in soil treated with nitrogen modified technical lignins was evaluated in a pot experiment in a green house extended over two growth periods and compared with urea and NOVIHUM™, the reference product. The N-Sucrolin (4.46 t/ha) resulted in a cumulative dry mass yield that was similar to NOVIHUM™ (4.68 t/ha), the reference product. The N-lignocellulosic residue resulted in a lower cumulative dry mass yield (3.75 t/ha), as a result of a lower N content due to its considerable cellulosic content. The N-calcium lignosulphonate on the contrary yielded 0.91 t/ha dry matter, which was only 19.4% that of NOVIHUM™ as a result of stunted growth. It also had high solubility due to its high sulphur content causing it to rise and precipitate on the soil surface, making it susceptible to surface erosion. The causes of the stunted growth, although not evaluated in this study, could be linked to phytotoxic effects due to its high reducing sugar content (mainly xylose).

6.1 Introduction

Physiological active substances which have negative effects in the growth and development of plants are sometimes present in N-modified technical lignins (Fiedler and Schmidt 1979). These result from the presence of a high proportion of reducing substances such as carbohydrates in the raw materials. Physical evidence of the presence of such compounds includes leaf necroses, shoot stuntedness, as well as death of the shoots. These effects were observed when poplar was grown with *N*-modified materials produced from sulphite spent liquors. In spruce and pine, irregular height and discoloration of the needles were observed (Fiedler and Schmidt 1979; Fiedler and Schmidt 1981; Fiedler and Schmidt 1983). These effects resulted from the formation of five membered nitrogen containing heterocyclic compounds during oxidative

ammonolysis. In general, sugar is known to form nitrogen containing heterocyclic compounds, mainly imidazoles and pyrazines when reacted with ammonia (Kort 1970). When Schiene et al. (1996) investigated the role of sugars on *N*-modified lignins derived from spent sulphite liquors, substances with basic lignin structures and monosaccharides in an aqueous system consisting of $^{15}NH_3/H_2O_2$ were found. Subsequently, the^{15}N-containing products were investigated by means of ^{15}N NMR. The spectra of all the products showed typical signals for NH_4^+, amide and amine nitrogens. Only the products from monosaccharides showed signals at -205.2 to -208.7 ppm interpreted for azole, and signals at -75.3 to 64.5 ppm interpreted for azole or azine and also for imine structures. It is in this regard that the content of reducing sugars present in the technical lignin is also a very important criterion for the selection of raw materials for oxidative ammonolysis.

The objective of this study was to evaluate N-modified, South African obtained, technical lignins for biomass production and to compare them with urea, NOVIHUM™ as well as an N-modified material prepared from Kraft lignin Indulin AT.

6.2 Materials and methods

6.2.1 Place of study

The performance evaluation was carried out in a green house facility on yellow mustard (*Sinapis alba*) extended over two growth periods at the Institute of Wood and Plant Chemistry, Technical University, Tharandt, Germany.

6.2.2 Soil and fertiliser

The properties of the N-lignins which were evaluated are shown in table 6-1 below. The N-lignins were prepared according to ambient pressure procedure patented by Fischer et al. (2000). The moisture content of the N-lignins and the soil was ascertained prior to application using TAPPI standard method T 211 om-85.

Table 6-1: Elemental composition, C/N ratios and sulphur contents of the N-lignins (values given on dry organic matter basis).

Product	Nitrogen [%] on oven dry basis*				Carbon	C/N	total S
	total	NH_4^+-N	NH_2-N	Sob-N	[%]	ratio	[%]
N-Sucrolin	**3.82**	46.6	9.4	44.0	63.8	16.7	0.22
N-Lignocellulosic residue	**2.66**	28.2	11.6	60.2	47.0	17.7	0.10
N-Lignosulphonate	**3.36**	52.7	11.9	35.4	42.0	12.6	4.79
N-Indulin AT	**3.07**	30.5	11.4	58.1	62.5	19.8	2.01
Novihum™	**5.55**	30.3	12.4	57.3	63.9	11.51	0.74

* The NH_4^+-N, NH_2-N and Sob-N were calculated as a percentage of the total nitrogen

Mitscherlich pots, 20 cm in height and a cross-sectional area of 283 cm^2 were filled with 7kg of a loamy sand (pH measured in $CaCl_2$ = 6.7, CEC = 3.0 meq/100g), each containing 1% (70g) of the *N*-modified products. The basic fertilisation data is given in table 6-2 below. The immediately plant available nitrogen (NH_4^+-N) of N-calcium lignosulphonate, N-lignocellulosic residue and N-Indulin AT were supplemented with urea (in separate variants) to bring them to 900mg N/pot, so that they had the same level of NH_4^+-N as the urea applied. Each treatment was replicated three times.

Table 6-2: Nitrogen content in the fertilising materials and dosage in the pots.

Material	N content in material [%]	Urea		N-lignin				Total Plant available N [mg/pot]
		N [mg/pot]	Kg N/ha	Dosage [% mass/pot]	Dosage [g/pot]	N [mg/pot]*	NH_4^+-N** [mg/pot]	
N-Sucrolin	**3.82**	-	-	1	70	2674	1246.08	1246.1
NOVIHUM™	**5.55**	-	-	1	70	3885	1177.16	1177.16
N-Calcium lignosulphonate	**3.36**	-	-	1	70	2352	663.26	663.6
N-Lignocellulosic Residue	**2.66**	-	-	1	70	1862	525.08	525.1
Urea	46	900	320	-	-	-	-	900
N-Calcium lignosulphonate -Urea	3.21	236.76	-	1	70	2588.76		900
N-Lignocellulosic residue-Urea	3.20	374.92	-	1	70	2236.92		900
N-Indulin AT-Urea	3.42	244.56	-	1	70	2393.56		900
N-Indulin AT	3.07		-	1	70	2393.56	655.45	655.45

Legend: The grey shaded area depicts the N-lignins after the addition of urea. The N-Indulin AT without urea was not evaluated. Its values prior to urea addition are given in the last row.

*The amount of nitrogen in each pot was calculated using the following equation:

$$\text{N (mg/pot)} = \frac{\%\text{ N content in material}}{100\%} \times (\text{dosage in g/pot}) \times 1000 \qquad (6\text{-}1)$$

**The total NH_4^+-N in mg per pot was calculated using the following equation:

$$NH_4^+ - \text{N (mg/pot)} = \frac{\%NH_4^+\text{-N per material (see table 5-2)}}{100\%} \times \text{N (mg/pot)} \qquad (6\text{-}2)$$

6.2.3 Seeding, tendering and processing

29 seeds were placed in each pot using a Perspex grid ensuring spacing of 25mm between the seeds. The plants were given 150ml of water every second day to compensate for water losses through evaporation and plant uptake. The heights of the plants were measured every 7 days with a ruler. When blossoming, the plants were harvested after 35 days by cutting the shoots 2mm above the surface. Dry matter yield was determined by drying the shoots at 70°C until constant weight. Total nitrogen in the plants was determined through CNS analysis. Protein content was determined by multiplying the total nitrogen by 6.5. After harvesting the plants at the end of the first growth period, new seeds were planted into the pots without additional nitrogen. They were tendered and processed as already discussed.

6.3 Results and discussion

6.3.1 Growth rate

The growth rates of plants treated with N-Sucrolin, N-lignocellulosic residue, N-lignocellulosic residue-urea were similar to those observed with NOVIHUM™, N-Indulin AT-urea and urea. However, as can be seen from figure 6-1 below, the N-calcium lignosulphonate and N-calcium lignosulphonate-urea treatments resulted in a slower growth rate. At the end of the first growth period, these plants had an average height of less than 10 cm; contrary to the average of about 28cm for the rest of the other variants. During the second growth period, a similar growth phenomenon was observed. Figure 6-2 below gives a visual impression of the differences in the growth phenomenon of the N-calcium lignosulphonate in comparison with the modified Sucrolin.

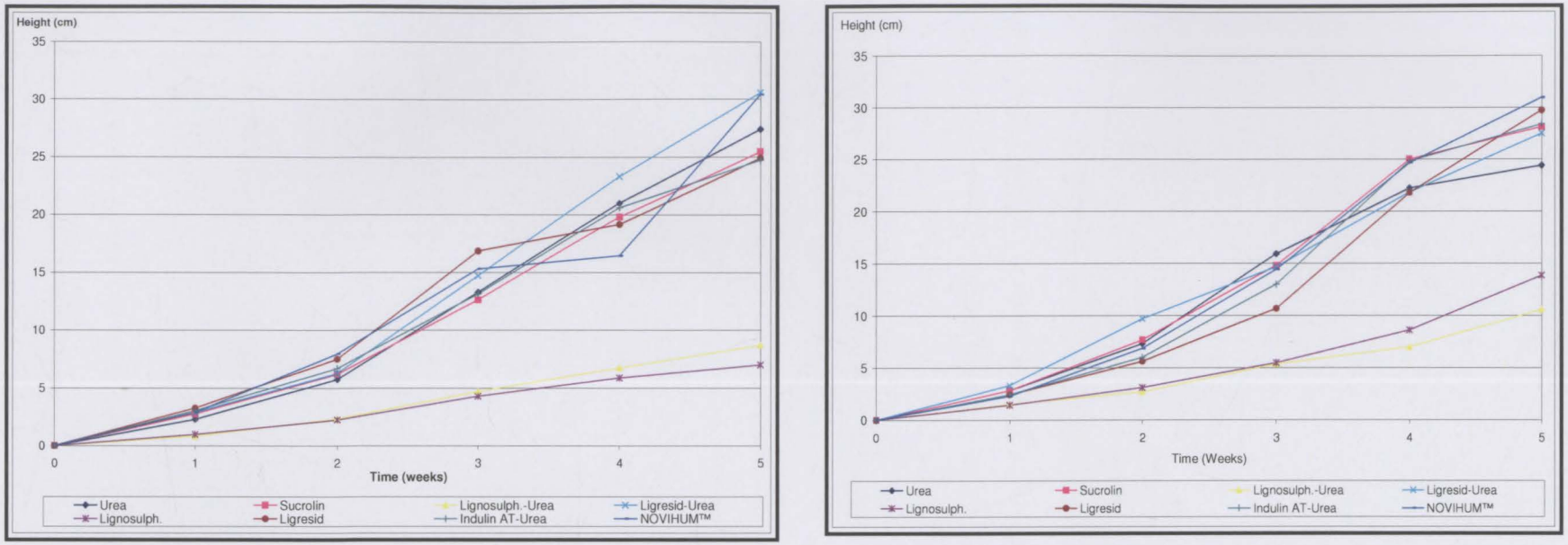

Figure 6-1: Growth rate in height of mustard plants fertilised with *N*-modified technical lignins, urea, and NOVIHUM™ during the first (left graph) and the second growth period (right graph).

Figure 6-2: Growth characteristics of yellow mustard (*Sinapis alba*) fertilised with N-Sucrolin (left) and N-calcium lignosulphonate (right).

Whether the results obtained are in agreement with what (Schiene et al. 1979) found when investigating the role of sugars in sulphite spent liquor during oxidative ammonolysis, is subject to further investigations. However, preliminary investigation in this regard showed that the calcium lignosulphonate had a high content of reducing sugars (mainly pentoses) (see chapter 4). In order to avoid the phytotoxic effects that may result from the presence of these compounds, the process of oxidative ammonolysis should be controlled in such a way that the formed heterocyclic compounds are incorporated in the macromolecular matrix (Fischer et al. 1990).

6.3.2 Dry matter yield

Figure 6-3 below presents the dry matter yield results of yellow mustard (*Sinapis alba)* over two growth periods. Figure 6-4 presents cumulative dry matter yield. The results (figure 6-4) showed that the soil treatment with *N*- Sucrolin had a similar effect on dry matter yield of yellow mustard (*Sinapis alba)* as NOVIHUM™. Given the fact that the N-Sucrolin tested had a lower nitrogen content (3.8%) than NOVIHUM™ (5.5%), this material could be considered very effective from the nutrient efficiency perspective.

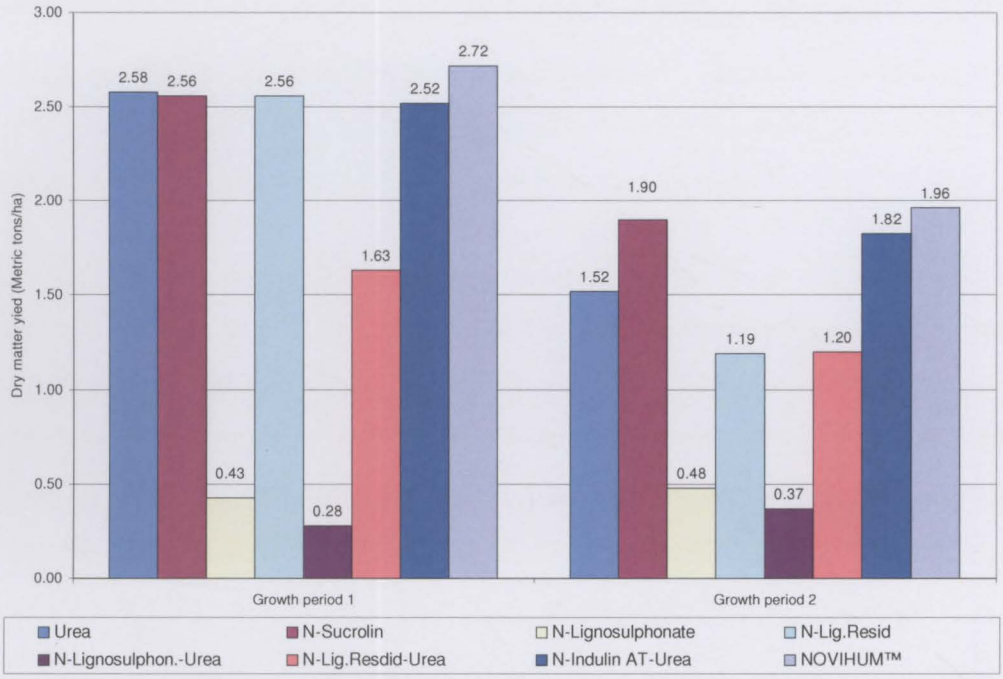

Figure 6-3: Dry matter yields of yellow mustard plants after fertilisation with N-lignins after two growth periods.

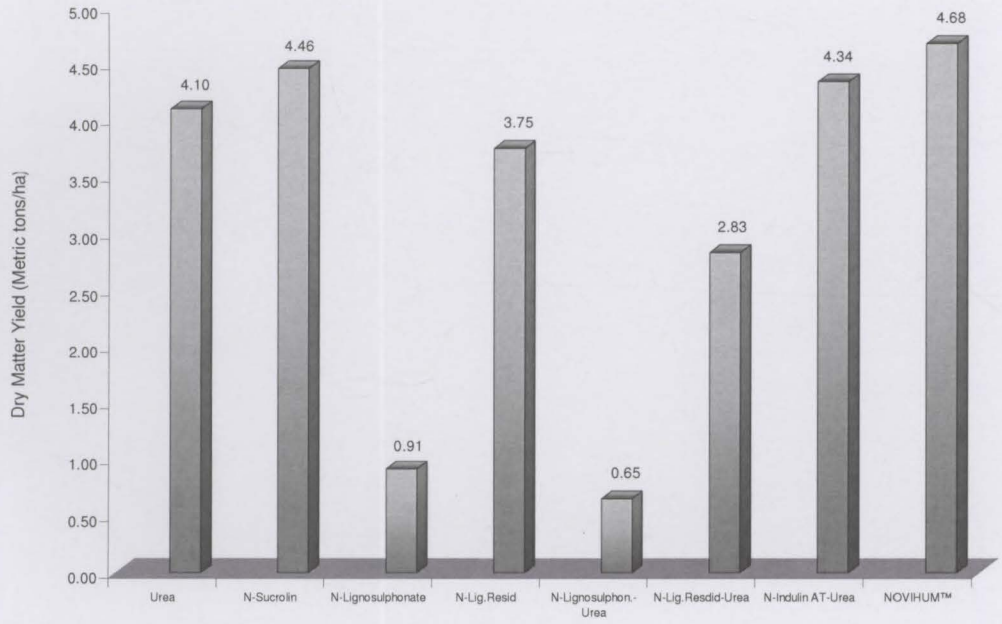

Figure 6-4: Cumulative dry matter yield of matter yields of yellow mustard plants after fertilisation with N-lignins after two growth periods.

Dry mass yields from N-lignocellulosic residue soil treatments were also comparable to N-Sucrolin and NOVIHUM™ in the first growing period. However, its lower nitrogen content (2.66%, see table 6-1) in comparison with N-Sucrolin resulted in reduced yield in the second growth period as well as overall yield (figure 6-4).

Ramirez-Cano et al. (2001) found that the use of urea due to its higher solubility and immediate availability to plants resulted in better growth in *Sorghum vulgare*. However, in this study, supplementing the N-content of N-lignocellulosic residue with urea only resulted in faster growth but lower biomass yield. Figure 6-5 below gives a visual impression of yellow mustard treated with N-lignocellulosic residue and modified lignocellulosic residue supplemented with urea. This could be attributed to excessive availability of nitrogen which does not necessarily lead to enhanced biomass yields.

Figure 6-5: Growth characteristics of yellow mustard (*Sinapis alba*) fertilised with *N*-lignocellulosic residue (left) and N-lignocellulosic residue-urea (right).

In contrast to other products, soil treatments with N-modified calcium lignosulphonate resulted in very low yields, even in combination with urea, indicating that nitrogen was not a growth limiting factor. In combination with possible phytotoxic effects, the material properties of the *N*-modified calcium lignosulphonate were not suitable for it to be used as a fertiliser i.e. the high water solubility of this product resulted in the material dissolving and precipitating on the soil surface after irrigation. After evaporation, a philistine salt crust formed which acted as a physical barrier during germination and early growth. Figure 6-6 below depicts the differences in the soil surface of soils treated with (from left to right) NOVIHUM™, N- calcium lignosulphonate, urea and N-Sucrolin. The dark colour of the soil surface in the calcium lignosulphonate treatment can be seen in the figure (second from left).

Figure 6-6: Soil treatments with NOVIHUM™, N-calcium lignosulphonate, urea and N-Sucrolin.

6.3.3 Nitrogen accumulation in plants

6.3.3.1 Total nitrogen

Figure 6-7 shows the accumulated nitrogen in yellow mustard (*Sinapis alba*) after two growth periods. In general, the NOVIHUM™ treatment resulted in the highest accumulation in nitrogen closely follwed by the N-Sucrolin. Nitrogen accumulation in all treatments except the N-calcium lignosulphonate, N-calcium lignosulphonate-urea and N-lignocellulosic residue-urea decreased in the second growth period, which was consistent with the decrease in nitrogen content in the soil. The lignocellulosic residue-urea treatmentresulted in less accumulated nitrogen than the one without urea, which showed that the nitrogen incorporated in the N-lignin in this material is better utilised than the inorganic nitrogen. The yellow mustard treated with calcium lignosulphonate also accumulated less nitrogen compared to other treatments which was also consistent with its dry matter yield.

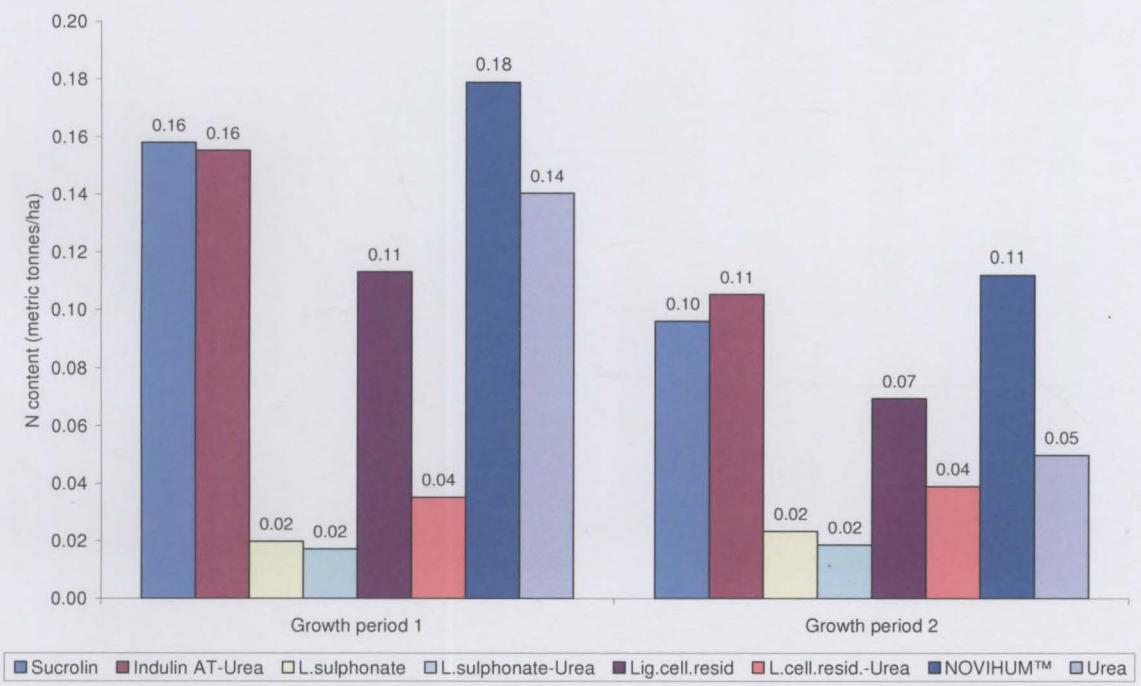

Figure 6-7: Total accumulated nitrogen in the foliage of yellow mustard treated with N-lignin materials.

6.3.3.2 Protein yield

The N-calcium lignosulphonate and the N-lignocellulosic residue had lower nitrogen contents (2.66% and 2.88% respectively) and hence yielded lower protein contents (figure 6-8). However, when these two materials were supplemented with urea, two different phenomena were observed; the protein yield resulting from the use of the N-calcium lignosulphonate increased by about 9.9% from 30.02 to 39.92%. On the contrary, the addition of urea to the lignocellulosic residue resulted in a speedy growth which was however not reflected in higher dry matter and protein yields. Only 14.40% protein was obtained when urea was added, contrary to 28.72% without it in the first growth period.

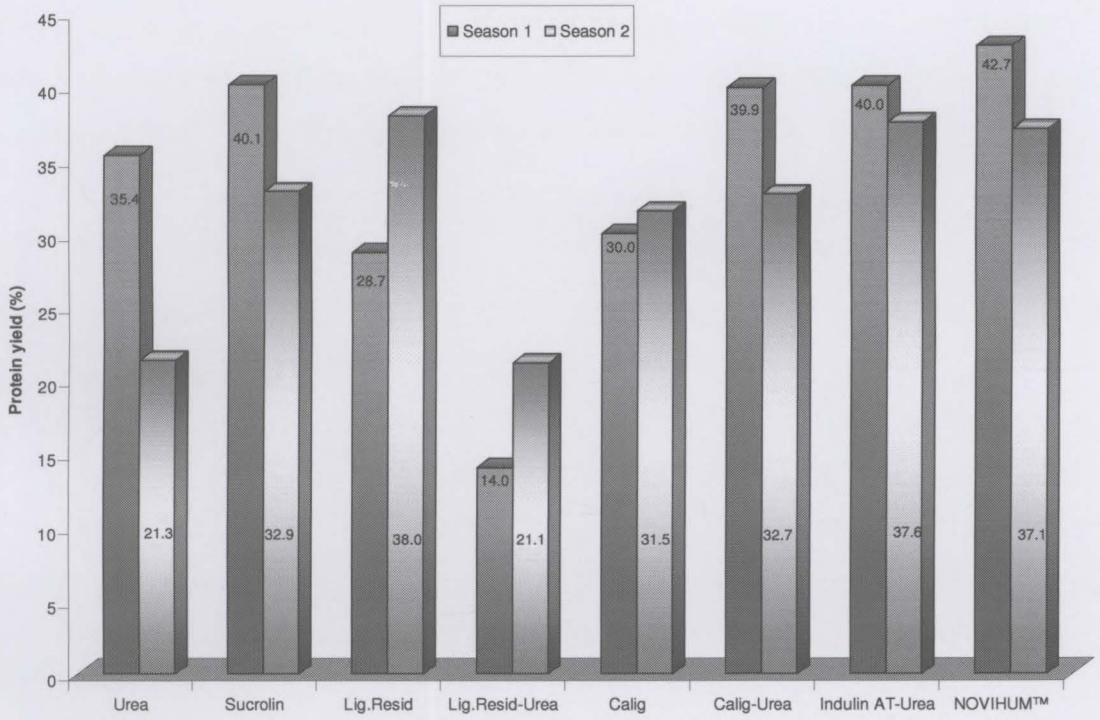

Figure 6-8: Above ground protein yield in yellow mustard plants over two growth periods.

In the second growing period, a general decrease in protein content was observed for all materials except the N-calcium lignosulphonate and the N-lignocellulosic residue. The

explanation for N-calcium lignosulphonate might be the fact that the plants consumed less nitrogen from the soil, which was associated with the slow growth.

6.4 Conclusion

The N-Sucrolin lignin yielded similar results to NOVIHUM™, proving that this material is suitable to be used as a source of nutrient for plants. The N-lignocellulosic residue showed similar yields as NOVIHUM™ and the Sucrolin in the first growth period. However, the yield decreased in the second growth period due to the lower nitrogen content. An additional source of nitrogen in the form of urea was not a good solution. Increasing the nitrogen content of the material through oxidative ammonolysis could be a better solution. The calcium lignosulphonate selected for the investigations seemed not to be a good raw material for preparing N-lignins as it showed retarded growth on plant tests. This could be associated with high content of reducing sugars (see chapter 3) and its high water solubility as a result of the pulping method.

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7.0 Phytohormonal content in relation to nutritional status of yellow mustard, *Sinapis alba* grown on soil fertilised with *N*-lignins

Abstract

Plant hormones abscisic acid and isopentenyladenosine were studied in the leaves of mustard seed, *Sinapis alba,* grown on soil fertilised with N-lignins prepared from South African technical lignins Sucrolin, calcium lignosulphonate and a lignocellulosic residue. Urea and NOVIHUM™, a patented product, were used for reference. Previous studies have shown that N-calcium lignosulphonate resulted in low biomass yield in comparison with other N-lignins prepared under the same conditions with similar N contents. The results of this investigation are discussed in relation to a possible role of absisic acid and isopentenyladenosine as indicators of mineral nutritional stress which could possibly result in the low yield. The abscisic acid contents showed a negative trend which did not indicate any relationship between the nutritional statuses in the leaves. A decrease in isopentenyladenosine was observed after the second harvest in comparison with the first harvest among all treatments consistent to what was expected. The results showed significant relationship between the different treatments i.e. the materials behaved in a similar manner. This indicated that the focus of future studies in the use of N-modified calcium lignosulphonate should focus on the suspected phytotoxic compounds which have been described by other workers as five membered, heterocyclic, nitrogen containing compounds.

7.1 Background

Plant hormones are extremely important agents in the integration of developmental activities as well as responding to the external physical environment. Environmental factors often exert inductive effects by evoking changes in hormone metabolism and distribution within the plant (Moore 1989). Of the commonly recognised classes of

hormones, cytokinins and abscisic acid will be discussed here in relation to the nutritional status of plants.

Cytokinins (CK) are N^6-substituted adenine derivatives that play a role in almost all aspects of plant growth and development including cell division, sink/source relationships, vascular development, chloroplast differentiation, apical dominance and senescence (Mok and Mok 2001). The most abundant types in plants are adenine type species which are adenines of substituted N^6 with either isoprene, a modified isoprene, a benzyl group or an *o*-OH-benzyl group. Cytokinins could be classified according to their physiological functions into active, transport, storage and inactivated forms. The free bases, isopentenyladenine (iP), *trans*-zeatin (Z) and dihydrozeatin (DZ) are thought to be active forms, and the ribosides, isopentenyladenosine (iPA), Z riboside (ZR), DZ riboside (DZR), the translocated forms. With respect to the structural similarity, Z and the derivatives, ZR and *trans*-zeatin riboside-5-monophosphate (ZMP), are grouped and generally referred to as 'Z-type cytokinins', and iP, iPA and isopentenyladenosine-5'-monophosphate (iPMP) are referred as 'iP-type cytokinins' (Takei et al. 2001).

The accumulation level of cytokinins is closely correlated with the nutritional status of the plant, especially mineral nitrogen (Kakimoto 2003). Endogenous cytokinin levels of the base/nucleoside fraction in leaves of *Betula pendula Roth.* were found to be significantly lower in the nitrogen-deficient plants than in the controls only 3 days after transfer to deficient conditions (Horgan and Wareing 1980). Kuiper et al. (1989) found that a transfer of *Plantago major ssp. pleiosperma* from concentrated nutrient solution to a dilute solution depressed the cytokinin concentration by 50% within two days of transfer. Takei et al. (2001) also found that upon addition of nitrogen to nitrogen depleted maize plants, iPMP started to accumulate in the roots within one hour preceding ZMP, ZR and Z. In the xylem flow, both exudation rate of xylem sap and the concentration of cytokinin increased. Recently, an excellent review of interactions between nitrogen and cytokinin in the regulation of metabolism and development has been given by Sakakibara et al. (2006). All these studies suggested that cytokinin metabolism and translocation could be modulated by the nitrogen nutritional status, and

that the resulting accumulation levels of cytokinin species in each tissue also could change.

Abscisic acid (ABA), also known as *abscisin II* or *dormin*, is a plant hormone which functions in many plant developmental processes including abscission and bud dormancy (Sengbusch 2003). ABA-mediated signalling also plays an important part in plant responses to environmental stress and plant pathogens. Like cytokinin, abscisic acid has been shown to be affected by mineral nutritional status of the plant. Studies by Battal et al. (2003) on the effects of different mineral nutrients on abscisic acid on maize (*Zea mays*) have shown that deprivation of micronutrients N, P, K, S and Fe caused an increase in the production of abscisic acid . Excessive concentrations of these macronutrients caused a decrease in ABA levels in all plant parts. Wallander et al. (1994) had also found earlier that ABA concentrations in lateral roots of *Laccaria bicolor* inoculated seedlings were positively related to N supply.

The foregoing discussion indicates that CK and ABA are inversely affected by the nutritional status of the plants i.e. in instances of nutritional stress CK in the plant decreases while ABA is increased. However, this phenomenon could also be time related i.e. it could be determined by the stage of the development of the plant. Shu-Qing et al. (2004) found that during the grain filling stage in rice, the declining instantaneous photosynthesis rate of leaves coincided with decreasing ZR and increasing ABA contents in both roots and leaves.

In our studies of oxidative ammonolysis of South African technical lignins, we found that one of the N-lignin products i.e. N-calcium lignosulphonate resulted in lower biomass yield despite having N contents in the same range as other N-lignins prepared under the same conditions (Liebner et al. 2006b; Tyhoda et al. 2007) (see also chapter 5). As the physiological mechanism underlying this low yield is not known, it was postulated that a measure of the concentration of the phytohormones discussed above, in the plants grown on soil fertilised with N-lignins prepared from South African technical lignins, would indicate whether there was a nutritional stress in the plants, which

resulted in the low yield. This chapter therefore reports on the changes of cytokinin isopentenyladenosine and ABA contents in the leaves of mustard seed *Sinapis alba* grown on soil fertilised with N-lignins prepared from South African technical lignins.

7.2 Materials and methods

7.2.1 Place of study

The study was carried out over two growth periods in an Ecotron (plant growth chamber NEMA GmbH, Netschkau, Germany) at the Institute of Plant and Wood Chemistry (IPC), Technical University of Dresden, Tharandt, Germany. The plant growth chamber was equipped with a temperature, moisture and radiation regulation system which was set over a 24 hour period to resemble a normal day and night weather system (see figure 7-1 below). The experiment was carried out over a five week period until blossoming.

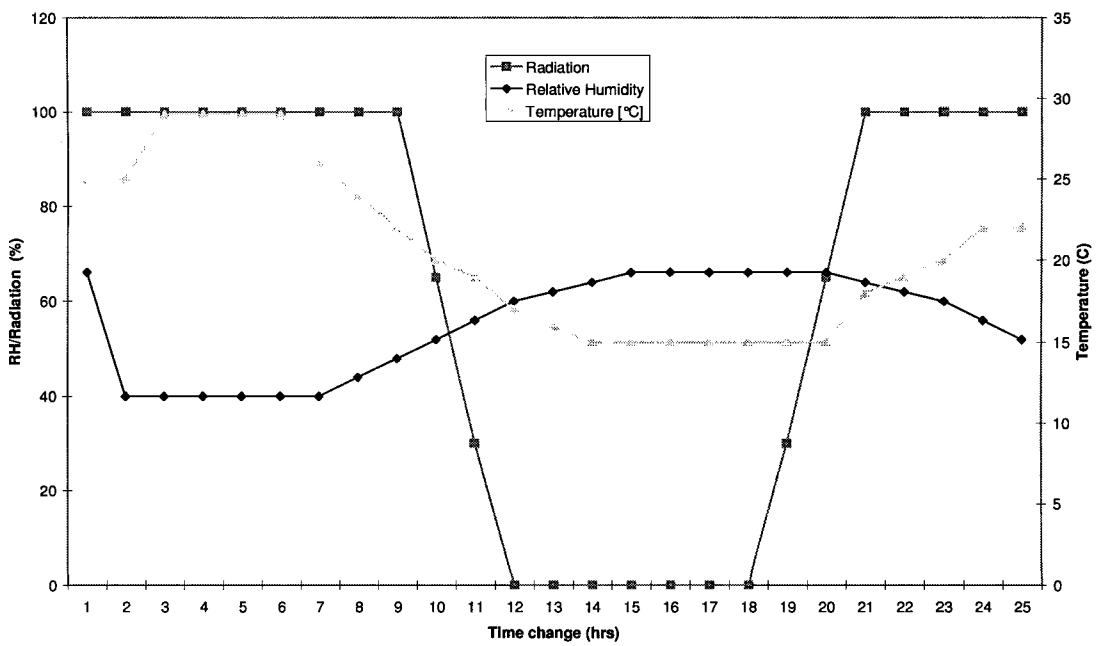

Figure 7-1: Daily environmental conditions in the Ecotron plant growth chamber.

7.2.2 Soil and fertiliser

The properties of the N-lignins used are shown in table 7-1 below. The N-lignins were prepared according to ambient pressure procedure patented by Fischer et al. (2000). The moisture content of the N-lignins and the soil was determined prior to application using TAPPI standard method T 211 om-85.

Mitscherlich pots, 20 cm in height and a cross-sectional area of 283 cm^2 were filled with 7kg of a sandy soil (pH measured in $CaCl_2$ = 7.0), each containing 0.5% (35g) of the *N*-modified products. The fertilisation data is given in Table 7-2 below. Potassium hydrogen phosphate (monobasic) was used as a source of potassium and phosphorus at dosages of 120 kg P/ha (4.24g K/pot) and 152 K/ha (5.35g P/pot) respectively. The urea was dissolved in water prior to addition to the pots. Each treatment was replicated three times.

Table 7-1: Elemental composition and C/N ratios of the N-lignins (values given on oven dry basis)

Product	**Nitrogen**				**Carbon**	**C/N**
	total % on oven dry basis	NH_4^+-N	NH_2-N	Sob-N	[%]	**ratio**
		(as a % of total N)				
N-Sucrolin	**4.95**	29.49	9.49	61.02	58.70	11.8
N-Lignocellulosic residue	**2.66**	36.57	14.92	48.50	48.50	17.80
N-Lignosulphonate	**3.36**	52.70	11.90	35.4	42.00	12.60
NOVIHUM™	**5.55**	30.3	12.4	57.3	63.9	11.51

Table 7-2: Nitrogen content in the fertilising materials and dosage in the pots.

Product	N content in material [%]	Urea		N-lignin			Total Plant available N [mg/pot]
		N [mg/pot]	Kg N/ha	Dosage [g/pot]	N * [mg/pot]	NH_4^+-N* [mg/pot]	
N-Sucrolin	**4.98**	-	-	35	1743	514.01	514.01
NOVIHUM™	**5.55**	-	-	35	1942.5	588.58	588.6
N-Calcium lignosulphonate	**3.36**	-	-	35	1176	331.63	331.6
N-Lignocellulosic Residue	**2.66**	-	-	35	931	262.54	262.5
Urea	**46**	580	206	-	-	-	580

*The amount of N and NH_4^+-N in each pot was calculated as described in chapter 6.

7.2.3 Seeding, tendering and processing

29 seeds were placed in each pot using a Perspex grid ensuring spacing of 25mm between the seeds. The plants were given 150ml of water every second to compensate for water losses through evaporation and plant uptake. When blossoming, leaf samples were collected from each pot and the plants were removed before new seeds were planted to start the second growth period.

7.2.4 Plant processing and analysis

7.2.4.1 Plant hormone analysis

Leaf samples were collected randomly from each pot and placed into polypropylene Falcon tubes. The samples were frozen in liquid nitrogen immediately after sampling and kept at -20°C before further processing.

Lyophilization: The leaf samples were further prepared by grinding into a fine powder using a Retsch MM 2000 Micro Dismembrator (ball mill). Prior to milling, the Teflon vessel and the agitate ball were washed with distilled water, dried and placed in liquid nitrogen for cooling prior to milling the samples. A pair of forceps was used to collect the sample from the polypropylene containers. An empty bottle was labelled, weighed and placed in liquid nitrogen. About 5g of the material was placed in the Teflon vessel as quick as possible to avoid the samples gathering moisture. Milling of the sample was carried out at a frequency of 100 swings per second for 2 minutes. The homogenized

sample was placed in the weighed and cooled bottle. The samples were then freeze dried at -20°C for 14 days. The decrease in mass was monitored on a regular basis to determine the dryness of the samples. When the mass was constant, the samples were stored at -20°C. The water content of the samples was determined.

Extraction: The lyophylizate was taken out after allowing the samples to reach room temperature. Approximately 100mg of the lyophylizate was weighed into a polypropylene Falcon tube after which 10ml of the extractive agent[3] was added. The extraction was carried out at 4°C in the dark for 4 hours with occasional shaking. The samples were centrifuged for 10 minutes at 3000 rotations per minute at a temperature of 4°C. 8ml of the solution was placed into a 25ml round bottom flask. The solvent was removed in a rotor vapour at 40°C under vacuum. 2ml of a TRIS-buffer solution[4] was added to the solution and shaken for one hour to resolve the hormones. The solution was taken into a 2ml – Eppendorf micro reaction tube and centrifuged for five minutes at 10 000 rpm at 4°C. The solution was split into two portions. 750µl of each portion was placed into a 1.5ml Eppendorf tube. The solution was stored at -20°C. The 750µl samples were used for the preparation of ELISA sample solutions.

7.2.5 Data presentation and statistical analysis

Data were summarised in Excel (2003, 1985 – 2003) package and analysed. The mean responses for all treatments were weighted with Statistica 7 computer package. After providing significant differences at $p \leq 0.05$, a one way Random Analysis of Variation (RAMNOVA) was used to test significant differences between specific variants. In cases where the data was not normally distributed, a nonparametric test i.e. Mann-Whitney U test was used. All the data was analysed at 95% level of significance.

[3] The extractive agent was prepared by adding 0.25ml triethyl amine and 0.575g of sodium dithiocarbamate into 250ml of analytical grade methanol.

[4] TRIS buffer solution was prepared by adding 25mM Tris (hydroxymethyl)-aminomethane in water and the pH was adjusted to 7.5.

7.3 Results and discussion

7.3.1 General observations

The growth characteristics of plants treated with N-Sucrolin were similar to those observed with urea and NOVIHUM™. Soil treatment with N-lignocellulosic residue showed a lower biomass due to the low nitrogen content. These phenomena were discussed in chapter 6. As also discussed in chapter 6, the growth pattern as well as yield of plant fertilised with N–calcium lignosulphonate resulted in a slower growth rate and low yield despite the marginal differences in N contents compared to other materials. Figure 7-2 below shows a similar phenomenon as observed in chapter 5 i.e. low biomass of N-calcium lignosulphonate.

Figure 7-2: A visual presentation of the growth characteristics of plants treated with (from left to right) NOVIHUM™, N-calcium lignosulphonate, N-lignocellulosic residue, N-Sucrolin and urea.

7.3.2 Abscisic acid

There were no significant differences ($p = 0.16$, Mann-Whitney U) in mean ABA content in the shoots after the first and the second harvests for all the treatments (figure 7-3). A bootstrap test for significant interactions (figure 7-4) also revealed that there

were significant differences in ABA content in the first and second harvests as well as among the different treatments.

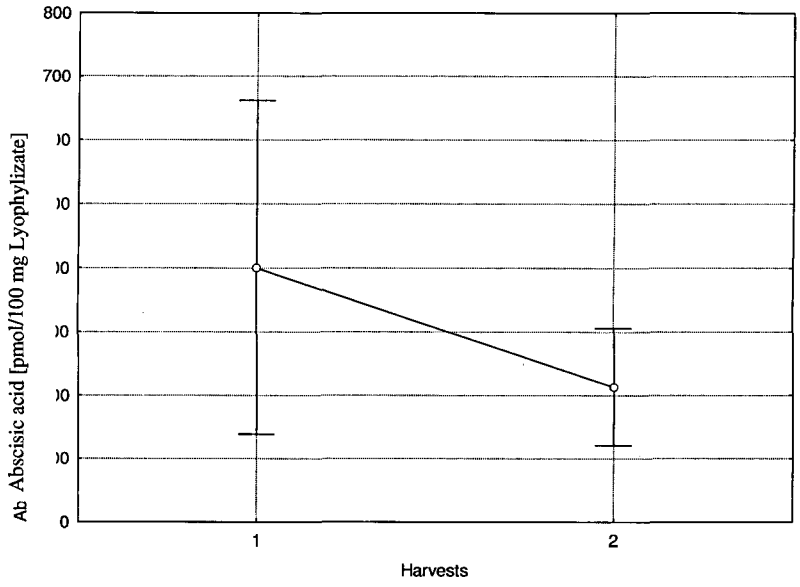

Figure 7-3: Weighted means of abscisic acid concentration after two harvests.

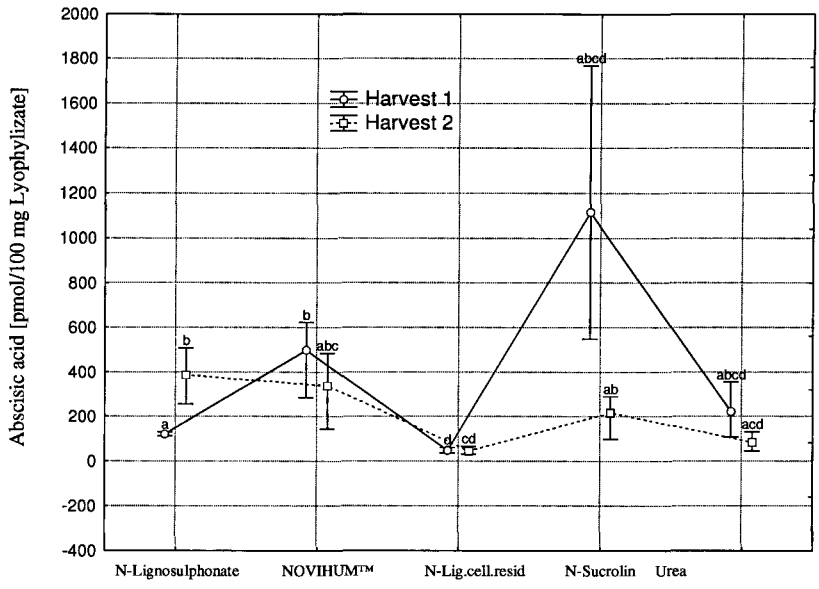

Figure 7-4: Bootstrap means of ABA after two harvests

7.3.3 Isopentenyladenosine

Figure 7-5 shows mean weighted values of isopentenyladenosine content among the different treatments after two harvests. The low content after the second harvest was consistent with the decrease in the content nutrients in the soil (Kakimoto 2003).

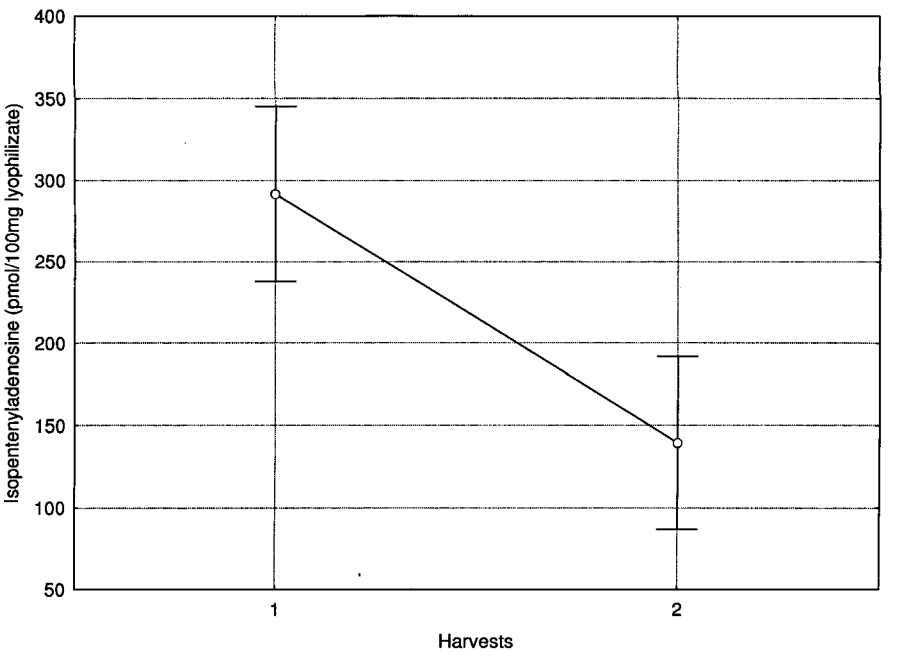

Figure 7-5: Weighted means of isopentenyladenosine concentration in the shoots of yellow mustard (*Sinapis alba*) after two harvests (Vertical bars denote 0.95 confidence intervals).

When the individual treatments were compared after two harvests (figure 7-6), no significant differences (p=0.09725, Mann-Whitney U) in mean isopentenyladenosine content were observed i.e. the materials behaved in more or less the same manner although differences could be expected due to the differences in nitrogen contents.

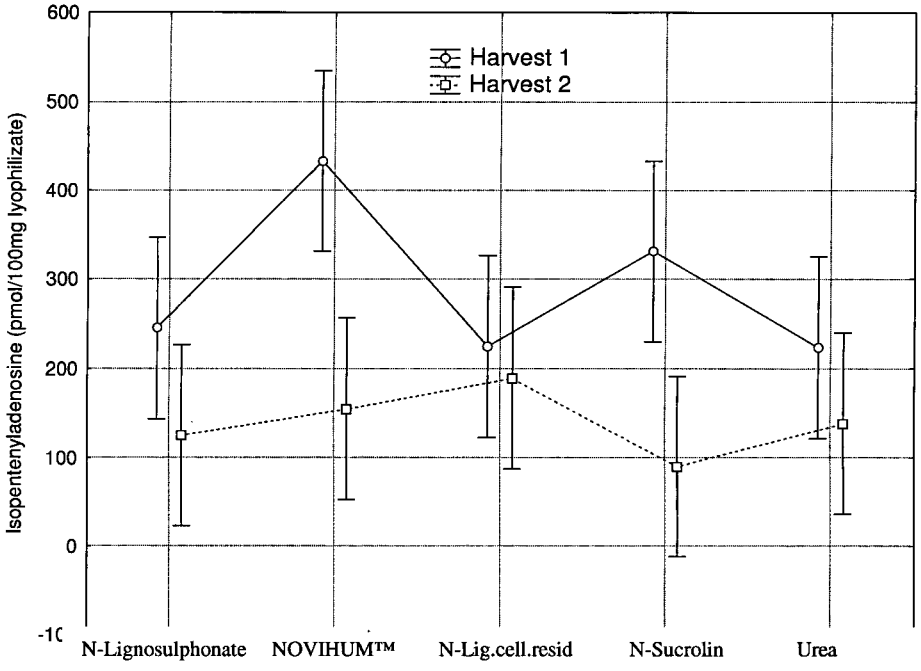

Figure 7-6: Comparison of the mean content of isopentenyladenosine among the different treatments.

7.4 Conclusion

A trend opposite to what was expected regarding the effect of the nutritional content of the materials as related to abscisic acid content was observed. A trend of decreasing isopentenyladenosine content was observed. However in most cases the differences were not significant indicating the similarity in behaviour of the N-fertilisers on a physiological basis. The results did not show any different behaviour in the N-calcium lignosulphonate treatment in comparison with other treatments which further indicates that the low yield observed with this treatment was not associated with nutritional status. Further studies on the use of N-calcium lignosulphonate should, therefore, focus on the specific or suspected phytotoxic compounds that result in low yield; described by other workers as five membered, heterocyclic compounds such as imidazoles. In addition, modifying the process conditions such that the xylose content of the raw material is drastically reduced should be investigated.

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8.0 Rye grass response as affected by nitrogen release from different NOVIHUM™ application rates

Abstract

NOVIHUM™, a slow nitrogen release, high grade humic material was tested on rye grass against ammonium sulphate to establish its release behaviour and its effect on nitrogen use efficiency on crops grown in a green house. The material was applied at rates of 0.3%, 0.7%, 1% and 2 % (mass %). Ammonium sulphate was applied at 120 kg N/ha (equivalent to 0.7% NOVIHUM™) split over two applications. Three soil types i.e. soils having loam, clay and sand textures were used for growing the rye grass. The results showed that nitrogen was most efficiently used in the 0.3-1% range. At higher rates, lower biomass yields were observed, especially in sandy soil due to the low survival rate resulting from the toxic effect of excess nitrogen. A 2% application rate resulted in higher losses of nitrogen due to leaching as observed in percolation water.

8.1 Introduction

The efficiency of any fertiliser or soil fertilising material depends mainly on maintaining sufficient nitrogen levels within the plant root zone throughout the growth and the development of a plant. However, due to processes such as degradation (chemical, photo-chemical and biological), volatilisation, leaching and immobilisation, the effective elements e.g. nitrogen, phosphorus, potassium, etc. in the fertiliser formulation often decrease too quickly. To offset this quick decrease, fertilisers are often applied in relatively excess dosages. In inorganic fertilisers, this sometimes causes environmental pollution through leaching of nitrates into ground water and too high concentration of nitrates in plants (Hadas et al. 1999; Costa et al. 2002). One of the solutions suggested to cope with the problem of nitrogen leaching into ground water from nitrogenous fertilisers is the incorporation of the fertilisers in controlled-release

formulations. The potential benefits of following this procedure as stated by Allen (1984) include:

i. a more efficient use of N by the crop
ii. lower toxicity resulting from excessive nutrient supply
iii. longer lasting N supply
iv. reduced volatilisation losses of N in the form of ammonia, and
v. reduced application costs.

However, the low cost effectiveness and limited recognition of the potential benefits to be gained from controlled release fertilisers (CRFs) or slow release fertilisers (SRFs) are so far the main reasons for their limited use. In addition, one reservation regarding the use of SRF/CRFs for reducing environmental problems related to fertiliser production is the extent to which the various materials used for preparing SRF/CRFs (plastics, formaldehydes, sulphur etc.) contribute to environmental pollution.

A different approach in the study and development of SRF/CRFs has developed over a period of more than 70 years. In an eco-friendly approach, lignins and similar carbonaceous materials have been used as starting materials to produce high grade humic materials with slow nitrogen release capacity. The benefits of such high grade humic materials have been detailed in numerous scientific publications. (Katzur et al. 2003) investigated the fertilising effects of NOVIHUM™ and the consequences of leaching losses to plant and the soil. Through pot tests and suction lysimeter tests, they established the amount of mineral fertiliser equivalent to the effective nitrogen of N-modified brown coal. In addition, the effects of NOVIHUM™ application rates on leaching were investigated on different mine dump substrates.

The current study investigates how much nitrogen is accumulated from NOVIHUM™ in crops over time (nitrogen use efficiency), as well as how leaching losses affect such accumulation. This was done using a simple and low cost intensive (in comparison with lysimeter tests) method pot test method according to Mikkelsen et al. (1994). Through

this method, not only the amount of nitrogen lost through leaching, but also the pattern of leaching could be monitored throughout the development of the plant.

8.2 Materials and methods

8.2.1 Place of study

The study was carried out in a green house facility at the Department of Forest and Wood Science, University of Stellenbosch, South Africa from the 5th of March 2005 until the 6th of September 2005. The green house was equipped with automated irrigation and cooling systems. It had 80% shade-netting side walls, polycarbonate roof end walls, 40% shade-netting ceiling and a concrete floor. The average monthly maximum and minimum temperatures in the green house over the experimental period is given in figure 8-1 below.

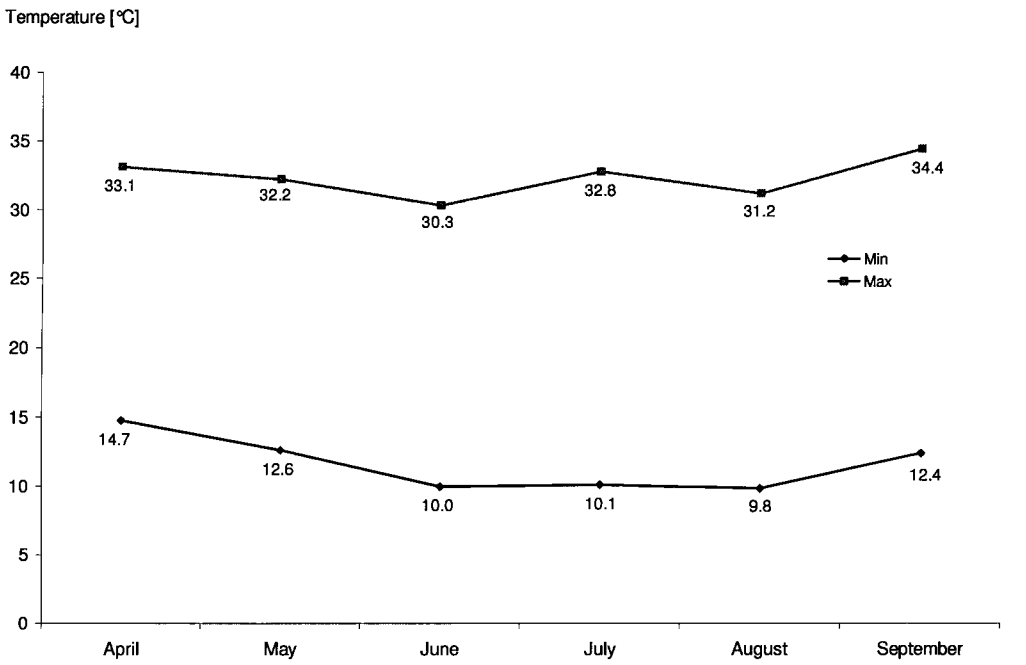


Figure 8-1: Monthly average minimum and maximum daily temperature variations in the green house over the experimental period.

8.2.2 Fertilisers and soils

NOVIHUM™ was obtained from Novihum GmbH, Großräschen/OT Freienhufen, Germany. The general properties of the material are given in table 8-1 below. The moisture content of the material was determined using TAPPI standard method T12wd-82 prior to application.

Table 8-1: General properties of NOVIHUM™

Shape	Granular (d_{90} 4mm)
Colour	Black
Compactness	$\approx 0.6 t/m^3$
Carbon	60-65 mass% (dry matter)
Nitrogen	5.5 mass% (dry matter)
Sulphur	0.6 – 0.7 mass% (dry matter)
Moisture content	About 20%
Ash content (%)	5-10 mass% (dry matter)
CEC_{pot}	30 – 60 mval/100g

Ammonium sulphate was obtained from Agricol, Brackenfell, South Africa and was used for comparison.

Three soil textural types i.e. sand from Malmersbury, clay from Koelenhof and loam obtained in the vicinity of Stellenbosch were used. The characteristics of the different soils are given in table 8-2 below.

Table 8-2: Properties of the three soil types used.

Soil sample	pH_{KCl}	**Ca [mg/kg]**	**Mg [mg/kg]**	**Na [mg/kg]**	**K [mg/kg]**
Sand	8.6	2603	73	142	598
Clay	8.1	2566	224	458	643
Loam	7.1	1965	162	241	1421

The soil and NOVIHUM™ were mixed prior to placing inside the pots. Phosphorus and potassium were added as a solution of KH_2PO_4 at rates of 120 and 152 kg/ha respectively. Ammonium sulphate (21% N, 24% S) was applied as a solution at a rate

of 120 kg/ha with 20 kg/ha being applied at sowing and 100 kg/ha after the first harvesting of rye grass.

8.2.3 Pot preparation

Plastic pots with a soil surface area of 324.16 cm^2 and a total volume of 4l were used. 24 perennial rye grass (*Lollium perenae*) seeds were planted 3cm apart at a depth of ca 0.6cm in each pot. The pots were sealed at the bottom with non-woven cloth (ex. Kimberly Clarke), so as to prevent soil loss from the pots. The test procedure used by Mikkelsen et al. (1994) to establish nitrogen leaching and uptake from CRFs was used. According to this method, a plastic funnel was placed at the bottom of each pot to collect the leachate draining from the pots into 2l plastic bottles. The pots were set on wooden strips, placed on a rack which suspended the tip of the funnel inside the 2L plastic bottles. 1ml of Ag_2SO_4 (which when diluted resulted in a minimum concentration of 1 $\mu g\ mL^{-1}$) solution was added to each bottle to inhibit microbial growth. Black plastic sheets were draped around the racks to shield the collection bottles from sunlight. Watering of the pots was done every second day using an automatic sprinkler system. The irrigation system was turned on for 4 minutes, which is equivalent to an average of 150ml of water applied per pot per irrigation. Due to the differences in the amount of water received by the pots in different zones, the pots were placed randomly and their position was changed once every week.

8.2.4 Experimental design

As shown in table 8-3, the study was designed as a five nitrogen application rates of NOVIHUM™ by three soil types factorial experiment, with 10 replicates. An application of only ammonium sulphate (21%N, 24%S) was prepared and used for comparison (also with 10 replicates). The experiment was carried out over a 26 week period and the rye grass was harvested after 43, 116 and 165 days.

Table 8-3: Nitrogen application rates.

	Control	$(NH_4)_2SO_4$*	NOVIHUM™			
		120kgN/ha	0.3 Mass %	0.7 Mass %	1 Mass %	2% Mass
Variants	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Loam	0g N/pot	378 mgN/pot	660 mgN/pot	1340 mgN/pot	2200 mgN/pot	4400 mgN/pot
Clay	0g N/pot	378 mgN/pot	660 mgN/pot	1340 mgN/pot	2200 mgN/pot	4400 mgN/pot
Sand	0g N/pot	378 mgN/pot	660 mgN/pot	1340 mgN/pot	2200 mgN/pot	4400 mgN/pot

*A total of 120 kg N/ha was applied over two periods during the experiment i.e. 20 kg N/ha (0.063gN/pot) at the beginning of the experiment and 100kgN/ha (0.315gN/pot) after the first rye grass harvest.

8.2.5 Data collection

8.2.5.1 Survival rate

The survival rate (in percentage) was expressed as the number of plants outgrowths that developed from the number of seeds that were planted.

8.2.5.2 Fresh mass determination

The fresh mass was determined by weighing the above ground plant material immediately after the plants had been cut at a height of ca. 3cm. This was done after the first harvesting.

8.2.5.3 Dry matter yield

After the plants were harvested, the samples were processed as follows:

- The plants were placed inside Kraft paper bags and dried in an oven at 70°C until constant weight. Temperatures above 70°C would cause thermal decomposition of the nitrogen (Thomson 1985).
- The dry matter yield was determined by milling the samples dried at 70°C in a Wiley mill. A sample was collected from the milled material and the moisture content was determined (at 103°C) using Tappi standard method T12wd-82. The value obtained was used as correction factor for the dry matter determined as at 70°C.

8.2.5.4 Nitrogen leaching

A pre-determined amount of water was added per pot to ensure that the final volume of leachate was 2L in each bottle. This was done immediately after cutting the plants. Total nitrate analysis in the leachate was carried out at Bem Lab, Somerset West, South Africa, according to Clescerl et al. (1998).

8.2.5.5 Tissue N-concentration

Tissue *N*-concentration was determined as NH_4^+-N as described by Forster (1995). The principle of the method is that ammonium cations react with salicyclate and hypochlorite in a buffered alkaline solution in the presence of nitroprusside to form a salicylic acid analogue of indophenol blue. The blue-green colour produced is measured at 660nm against calibration standard solutions with a spectrophotometer. A complexing agent is used to remove interfering polyvalent cations. The procedure is similar to the original indophenol blue method but substitutes the toxic phenol dye by its salicylic acid analogue.

Reagents

Digestion mixture

Chemicals were obtained from Merck Laboratories, South Africa. The digestion mixture was prepared by adding 0.42g of selenium powder and 14g of lithium sulphate to 350ml of 30% hydrogen peroxide. Concentrated sulphuric acid was added slowly under running cold tap water (ice was used when it was available).

Colour reagent

34g of sodium salicyclate, 25g of sodium citrate and 25 g sodium tartrate were dissolved in 750ml distilled water. 0.2g sodium nitroprusside was added and the volume was made up to 1L.

Alkaline hypochlorite buffer solution

The alkaline hypochlorite solution was prepared by adding sodium hydroxide and normal household sodium hypochlorite (a correction was made such that 5% available chloride was added) to a volumetric flask.

Spectrophotometric calibration solutions

4.719g of dry ammonium sulphate was dissolved in 400ml distilled water in a 1L volumetric flask and made up to volume (1000mg/L stock solution). 0.5; 1; 1.5; 2.0 and 2.5 ± 0.001 ml of the stock solution was pipetted into 100ml volumetric flasks to give working standards of 05; 10; 15; 20 and 25 mg NH^{+}_{4} nitrogen/L.

Digestion

Approximately 0.2g of rye grass sample material was accurately weighed into the digestion tube. 4.4ml of the digestion mixture was added to each tube. Six blanks were also prepared by adding 4.4ml of the digestion mixture into empty digestion tubes. The digestion was carried out at 360°C for 2 hours. After cooling, the digest was transferred into a 100ml volumetric flask with about 50ml distilled water and allowed to cool again. The flask was filled up to volume.

Analysis

A 0.1ml aliquot of the sample or calibration standard solution was transferred into a test tube. 0.5ml of the colour reagent was added and the contents shaken well. It was allowed to stand for 5min after which 5.ml of the hypochlorite solution was added. The colour was allowed to develop for one hour. Sample concentrations (C) were corrected by subtracting the average blank concentrations from each sample. The % nitrogen was calculated using the following equation:

$$\%N = \frac{C \times 0.1}{Sample\,mass\,(g)} \qquad (8\text{-}1)$$

where C is measured in milligrams per litre and a factor of 0.1 is used for conversion into percentage nitrogen.

8.2.5.6 Root shoot ratio

At the end of the experiment, four of the pots from each variant were emptied and the roots were separated from the soil by soaking the pots in water for few minutes before the soil was washed from the roots. The roots were air dried in a fume hood overnight to evaporate the wash water. The dry matter yield was determined in the same manner as was done for the grass stalks (see section 8.2.5.3).

8.3 Results and discussion

8.3.1 Survival rate of rye grass outgrowths

Figure 8-2 below shows the percentage number of plant outgrowths that survived in relation to the number of seeds that were planted per pot.

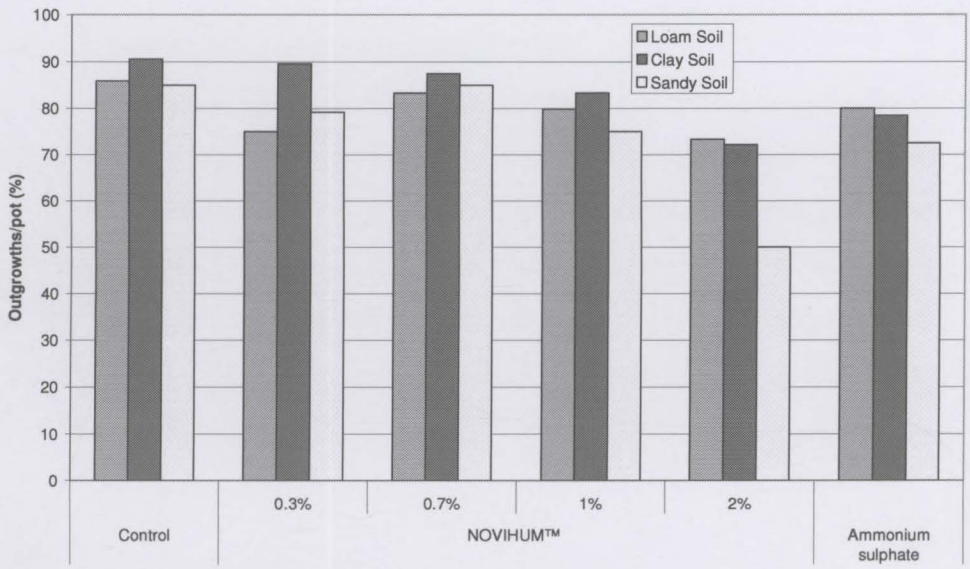


Figure 8-2: Survival rate of rye grass outgrowths.

As the figure shows, for all the variants, more than 70% of the plants survived. However, only about 50% of the plants survived at 2% NOVIHUM™ application in sand. This was mainly attributed to the toxic effect of the excess nitrogen that was applied. This phenomenon was also observed by Katzur et al. (2002) on studies on NOVIHUM™. Shaviv (1996) also observed this growth phenomenon with some SRFs

and explained it in terms of the high initial burst (more applicable in coated fertilisers) and tailing effects. The burst effect induces both high losses and in extreme cases induces damage to seedlings and young plants. The tailing effect causes a situation in which N supply is lower than plant demand. However, this phenomenon was not as pronounced in loam and clay soils. The low survival rate could also be attributed to the relatively high pH of the sand (see table 8-4) which could also have had an effect on the immobilisation of nutrients in the soil as also found by Prasad and Sinha (1982).

Table 8-4: Soil pH values

Soil type	Control	$(NH_4)_2SO_4$	0.3% NOVIHUM™	0.7% NOVIHUM™	1% NOVIHUM™	2% NOVIHUM™
Sand	8.12	8.43	8.48	8.60	8.35	8.56
Clay	8.16	7.93	7.90	7.96	7.84	7.89
Loam	7.40	7.90	7.89	7.94	7.67	7.47

8.3.2 Fresh mass yield after the first harvesting

Figure 8-3 below shows the average fresh mass yield of rye grass per pot after the first harvesting. As the figure demonstrates, an increase in fresh mass was generally observed with NOVIHUM™. The ammonium sulphate treatment was not better than the control. The highest fresh mass was achieved with loam soil at 2% application rate of NOVIHUM™. However, the average fresh mass yield per pot at the same rate was more than 2 times less in sand than in loam, which was consistent with the low survival rate observed and discussed above.

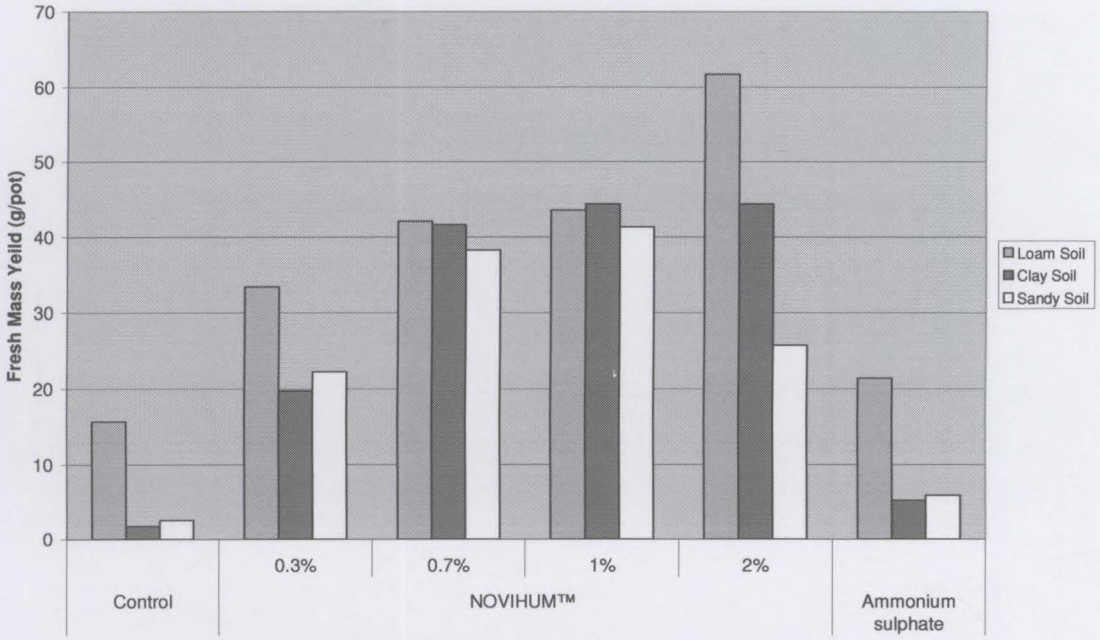


Figure 8-3: Average fresh mass yield after the first cutting per pot

8.3.3 Dry matter (DM) yield

Figure 8-4 below compares the total DM yields among the different NOVIHUM™ application rates in the first, second and third harvests, as well as the combined DM yield after three harvests. A general soil type effect could be observed with sandy soil giving the lowest yield. Consistent with the survival rate and fresh mass yields, a decline in yield was observed in sandy soil with increasing nitrogen application (>1% NOVIHUM™) as compared to loam and clay soils, especially after the first harvest. The yield after the first harvesting in ammonium sulphate was lower than at 0.7% NOVIHUM™ but it increased after additional nitrogen was applied after the first harvest.

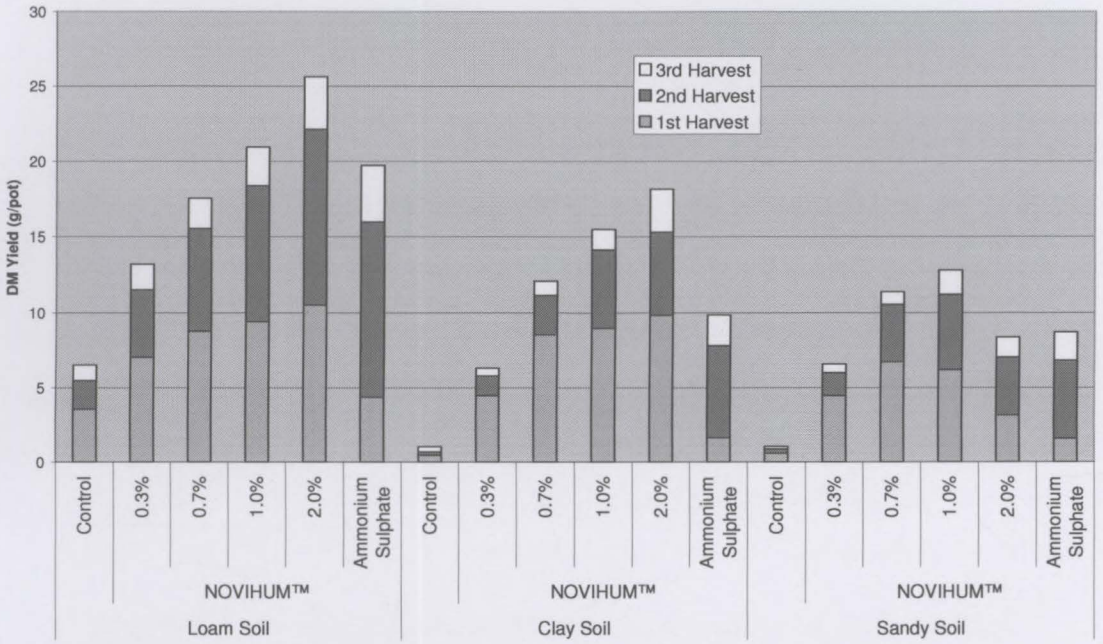


Figure 8-4: The accumulated average dry matter yield of rye grass after the first, second and third harvests

8.3.4 Nitrogen accumulation in plants

Figure 8-5 shows the total accumulated nitrogen after three rye grass harvestings. As the figure shows, nitrogen accumulation in sandy soil and somewhat in clay soil decreased with increasing amount of nitrogen applied per pot, which was consistent with the fresh mass and dry matter yields. The same phenomenon was observed with the DM yield as discussed in the above section. At effective equivalent nitrogen Katzur et al. (2003) observed that nitrogen accumulation was higher with NOVIHUM™ than from mineral fertiliser. The same phenomenon was observed here (0.7% NOVIHUM™ compared to the ammonium sulphate treatment) as the figure above demonstrates. This clearly demonstrated the success of NOVIHUM™, with loam soil (loam soil also had a lower pH) yielding the best results.

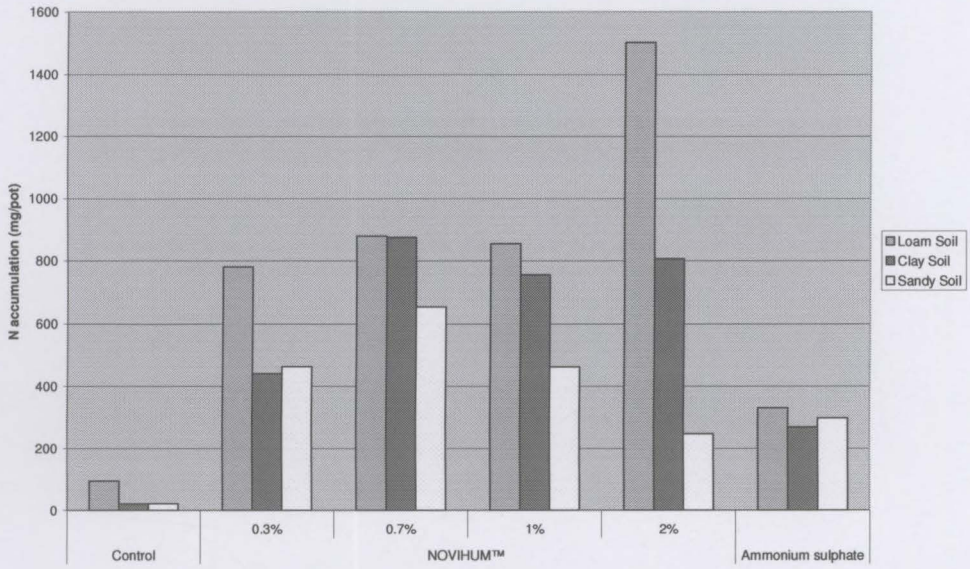
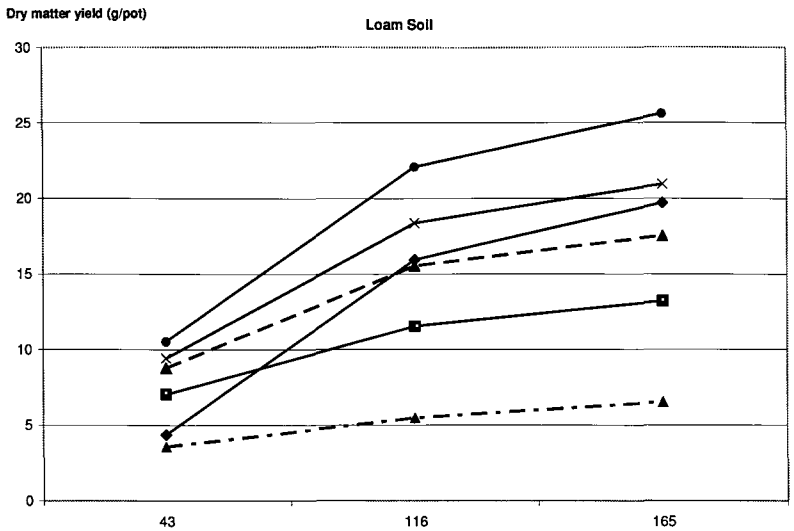
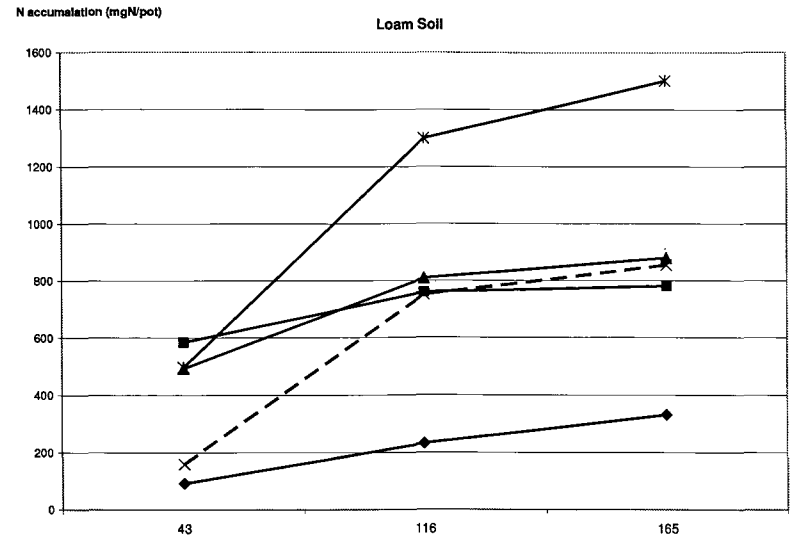
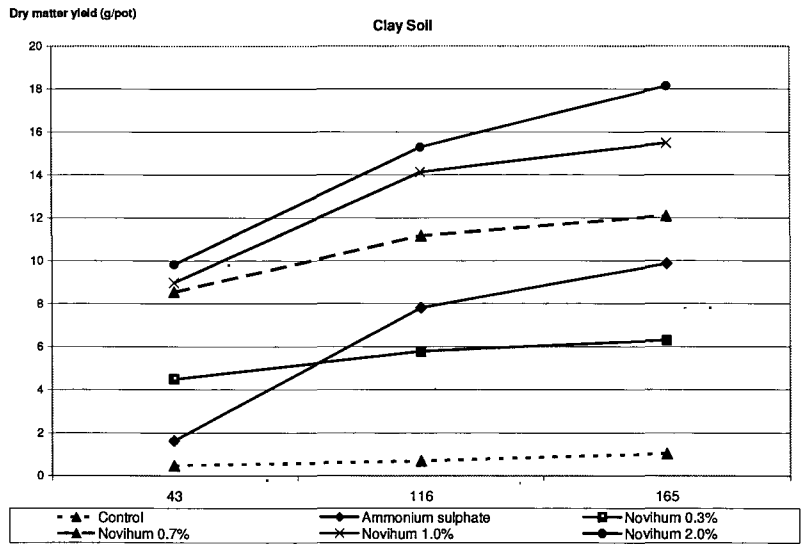


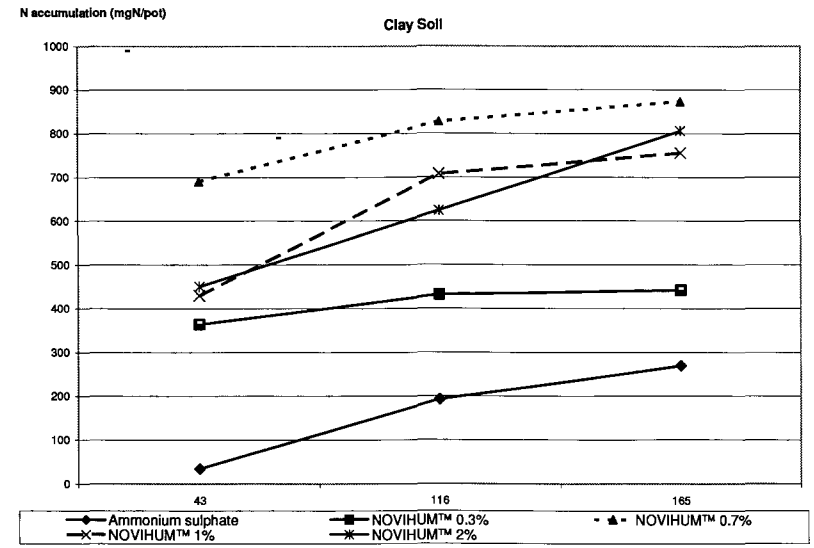
Figure 8-5: Total N accumulated in rye grass shoots after three cuttings

Figure 8-6 below relates dry matter yield and nitrogen accumulation in the shoots after three cuttings. As the figure shows, the highest dry matter yield was obtained with the highest nitrogen application rate (2%) in loam soil as well as the highest nitrogen accumulation in the shoots. However, as figure 8-7 below demonstrates, this was not necessarily the highest nitrogen use efficiency. Even though the highest fresh mass was also recorded with this soil, less than 40% of the nitrogen was accumulated in the shoots.









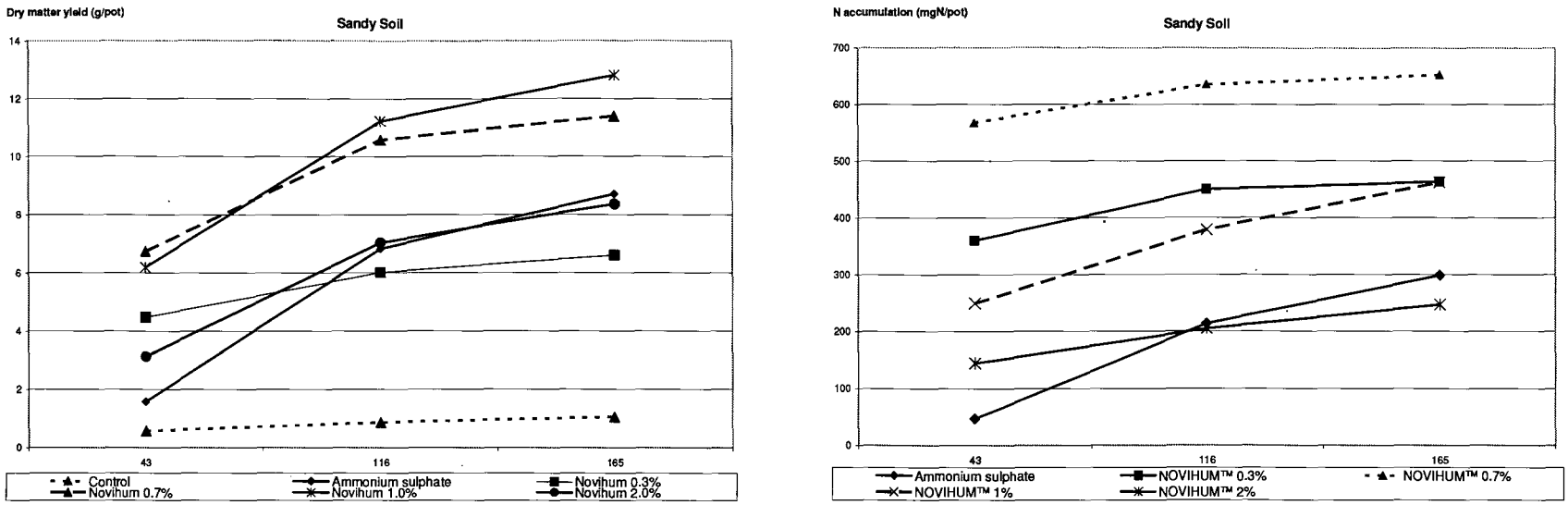


Figure 8-6: Dry matter (left) and accumulation of N in the shoots with time in days.

The less than 20% accumulation of nitrogen in the shoot at the 2% application rate in sandy soil is also quite remarkable and comparable to the value obtained by Katzur et al. (2003). This indicates that in as much as a higher application rate (2%) gives a higher yield; a more efficient rate is much lower than that. Inversely, a 0.4% application rate gives 80% use efficiency relative to the 2% application rate. However, to strike a balance between efficiency and yield, a 0.7% to 1% is tolerable.

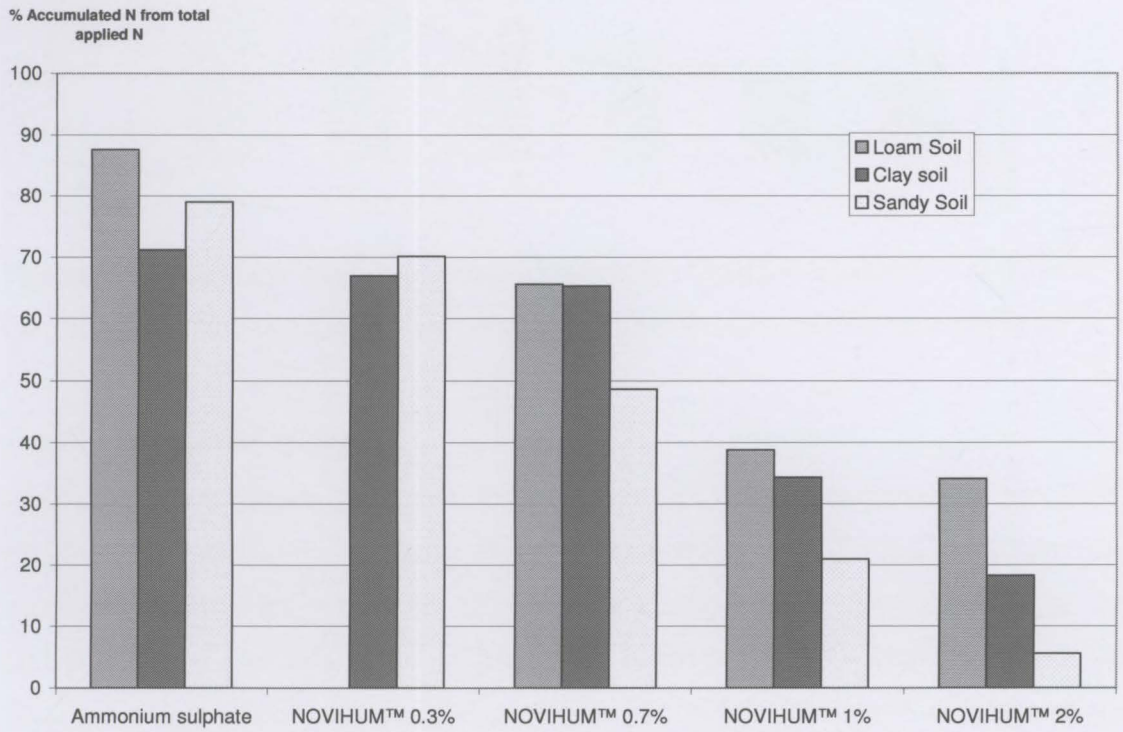


Figure 8-7: Nitrogen use efficiency in the shoots after 165 days as a percentage of the total applied N.

8.3.5 Root shoot ratio

The ratio of the dry mass yield of the roots to the dry mass yield of the shoots is given in figure 8-8 below. The figure illustrates that at low N levels, the plants in the control allocated more nutrients to the roots. However an upward trend is also noticeable in sandy soil as the nitrogen application rate increases. This could be attributed to low nitrogen availability (even though a huge amount was applied) due to the high pH.

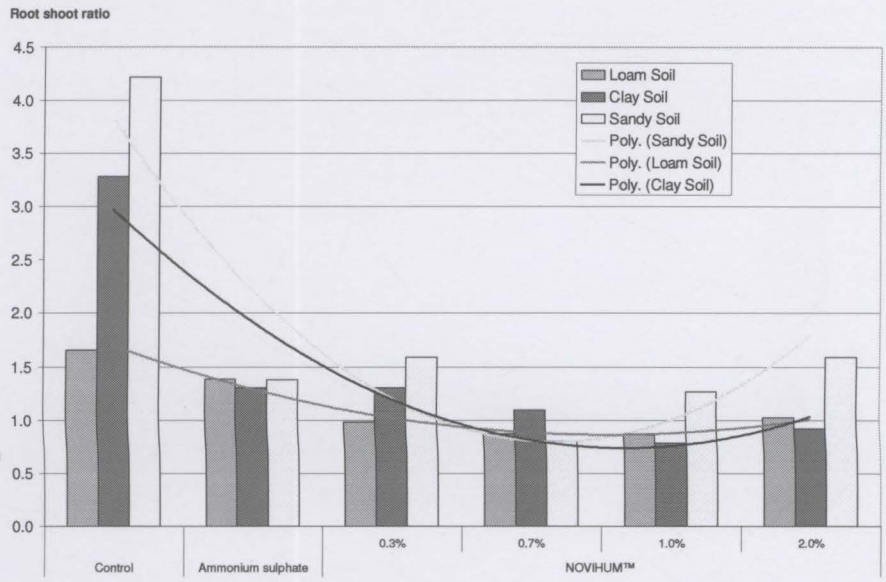


Figure 8-8: Dry mass yield ratio between the roots of rye grass measured at the end of the experiment and cumulative dry mass yield measured through out the experiment.

8.3.6 Leaching losses

Figure 8-9 below shows cumulative losses of nitrogen after three cuttings of rye grass. As the figure demonstrates, the amount of N leached at 0.3% and 0.7% nitrogen application rates were negligible. The highest leaching losses were observed at the highest nitrogen application rate i.e. 2%. Most of the nitrogen losses (more than 90%) occurred after the first cutting of rye grass from the NOVIHUM™ variants, whereas in ammonium sulphate they occurred mainly after the second and third harvestings due to the split nitrogen application. Losses with ammonium sulphate in sand and clay soils were higher than the effective equivalent nitrogen application rate 0.7% NOVIHUM™ due to the solubility of the material.

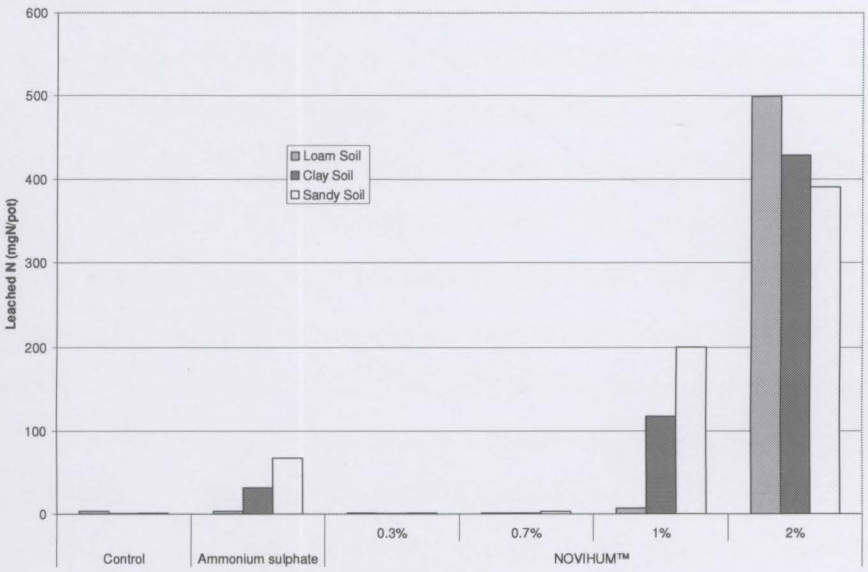


Figure 8-9: Cumulative leaching losses of nitrogen after three cuttings.

Studies by Katzur et al. (2003) were inconclusive regarding the pattern of leaching of nitrogen in two types of sand. However, the values they obtained on nitrogen obtained in the leachate were substantially lower than those allowed in drinking water. The leaching of N as affected by soil type could not be established as the loam>clay>sand trend was reversed at 2% NOVIHUM™. However, higher than the 1% application rate, the values could not be regarded as important as the nitrogen use efficiency at this rate is very low and the excess nitrogen also results in toxic effects on crops, especially in sand. In addition, at effective equivalent nitrogen, i.e. at 0.7% NOVIHUM™, the yield was slightly higher than in ammonium sulphate, consistent with observations by Katzur et al. (2003) and the nitrogen in the leachate was very low or negligible as compared to ammonium sulphate. According to the current study, at 1% application rate of NOVIHUM™, 200mg NO_3^- in the leachate obtained from sand was very high. However, this could also be regarded as a soil type effect as the losses in loam soil were quite low as compared to sand.

8.4 Conclusion

The effect of acceptable application rates of NOVIHUM™ was established on the three selected soil types. Too high application rate results were consistent with studies by other workers i.e. it results in excess nitrogen which is susceptible to leaching and in toxic effects to the plant. At effective equivalent nitrogen (0.7%), NOVIHUM™ resulted in better yield and lower nitrogen leaching than ammonium sulphate, consistent with studies by Katzur et al. (2003) with other mineral fertilisers. At a rate higher than 0.7%, the effect of the soil type should be considered. In addition, the nitrogen use efficiency at such rates should be taken into account as it decreases with increasing nitrogen application. It should also be considered that a higher rate of nitrogen application results in a higher mortality rate leaving fewer plants to make use of the nitrogen, and hence leaving it susceptible to leaching or released into the atmosphere as ammonia. Lysimeter tests are useful in establishing the release patterns of nutrients in the soil. However, the method used by Mikkelsen et al. (1994) used in this study to establish the nitrogen leaching and uptake from CRFs could be considered as a useful alternative for the study of lignin/lignite based slow release nitrogen fertilisers in the nursery due to its low material input required as compared to large scale lysimeter tests.

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9.0 Growth and nutritional response of tree seedlings on acidic and alkaline soils fertilised with N-lignins

Abstract

The performance of N-Sucrolin in growing tree seedlings in a soil rehabilitation effort of two distinctly different nutrient deficient soils i.e. an acidic soil resembling the situation found in post-mining landscapes and an alkaline soil, was evaluated against the patented reference product NOVIHUM™ and the conventional inorganic fertiliser ammonium sulphate in a nursery environment. *Eucalyptus marcarthurii*, a non-nitrogen fixer and *Acacia mearnsii* a nitrogen fixer were used as test species. All the fertilising materials could not mitigate the effects of the high pH (9.0) in the alkaline soil and consequently growth of the tree seedlings was not successful. In the acidic soil, the growth pattern of *Acacia mearnsii* was distinctly different from *Eucalyptus marcarthurii*, the non-nitrogen fixer. The response of *Eucalyptus marcarthurii* in soil treated with N-Sucrolin was slightly lower in terms of root collar diameter and dry mass yield than that of NOVIHUM™ treatment. However, differences were not significant. At equivalent nitrogen, the N-Sucrolin and the NOVIHUM™ had considerably higher dry mass yields and higher root collar diameters than ammonium sulphate due to the susceptibility of ammonium sulphate to leaching. Contrary to this, the N-lignins slowly released their nitrogen into the soil. The response of *Acacia mearnsii* in soil treated with N-lignins decreased. The N-Sucrolin yielded significantly lower root collar diameter and dry matter compared to the NOVIHUM™. Both the N-Sucrolin and the NOVIHUM™ improved the growth of tree seedlings, however, low application rates (0.5-1%) are recommended. This would even be more so for *Acacia mearnsii* due to its ability to fix its own nitrogen.

9.1 Background

Habitat transformation by humans is one of the major threats to biodiversity, and results in a conflict between the resource needs of an ever-increasing human population and the 'rights' of natural systems to exist and function (Anonymous 2006). One of the most severe forms of transformation is open-cast mining which causes almost complete destruction of terrestrial ecosystems. During opencast coal mining for example, the removal of top soil produces overburden materials and its pilling on the un-mined land creates overburden dumps. These dumps are physically, nutritionally and biologically poor in nature (Cornwell and Stone 1968; Wali 1987; Hüttl 1997; Zier et al. 1999; Lin et al. 2005). A key factor influencing the low fertility is a decrease in soil organic matter caused by loss or dilution of topsoil or mineralisation of organic carbon from topsoil stockpiles (Rate et al. 2004). A comparative study between mine rehabilitated soil and natural soil in Namaqualand, in South Africa revealed that mining operations result in the formation of saline sand tailings, stripped of a large portion of clay and organic matter fraction (Prinsloo 2005). Mining activities also result in soil acidification which is induced by oxidation of metal sulphides contained in the mine spoils (Thomas 1996; Hüttl 1997; Chabbi and Rumpel 2004; Lin et al. 2005). This reduces natural colonization of vegetation in the mine site which in turn may result in severe soil erosion.

In situations where topsoil has been lost or has become degraded such as in post mining landscapes, organic amendments such as sewage sludge can be used. Sewage sludge is rich in organic matter and nutrients and hence can be successfully used in agricultural practices, especially in arid countries such as South Africa (Hoffman 2000). It can be incorporated into soil as a soil conditioner and hence improve the physical, chemical and biological properties of soils which may enhance crop growth. It may be a useful source of trace elements to plants if the concentrations of these elements are low in unamended soil (Rate et al. 2004).

While these benefits can be exploited with treated[5] sludge, untreated sludge poses a lot of problems to the environment as a result of the multitudes of potentially toxic compounds which it contains, including among others chlorinated hydrocarbons, polynuclear hydrocarbons, nitrates and heavy metals (Chale-Matsau 2005).

In light of the foregoing discussion, organic based pollutant free, soil fertilising materials such as nitrogen modified technical lignins/lignites (N-lignins) can be used as soil amendments. N-lignins can be prepared through an ambient pressure based oxidative ammonolysis process patented by Fischer et al. (2000). The commercial product obtained from this process is trade named NOVIHUM™. It has unique properties as a high-grade humus and long-lasting, organo-mineral nitrogen fertiliser and has been successfully tested in rehabilitation activities and initial greening of problematic sites around the world (Liebner et al. 2006).

This study was aimed at evaluating the potential of N-Sucrolin for soil rehabilitation of saline (as found in Namaqualand) and acidic (as found in most post mining landscapes) soils. These soils, as discussed, are normally devoid of macro nutrients such as nitrogen and organic matter necessary for the growth and development of plants. Through application of carbon from the lignin, organic matter of these soils could be increased. In addition, the nitrogen chemically bonded to the technical lignins could be slowly released into the soil and continue to supply this macro nutrient over an extended period of time. *Acacia mearnsii,* a fast-growing, nitrogen-fixing tree adapted to a wide range of sites from the temperate and subtropical lowlands to tropical highlands, was used. It is also widely used for soil rehabilitation due to its ability to increase soil nitrogen levels (caused by the nitrogen fixing bacteria that live in symbiosis with leguminous plants) in many places around the world. It is also considered an alien invasive plant species in South Africa and other parts of the world. However, it has been found that rehabilitation of sites (following the removal of alien plant species) would sometimes be

[5] There are various methods available for sewage sludge treatement including aerobic digestion, air drying, anaerobic digestion, composting, lime stabilization and thermal treatment.

needed in order to prevent or reduce the soil erosion stimulated by the clearing of plants. In this regard, *Eucalyptus marcarthurii,* a commercial tree species which is tolerant to harsh environments was used for comparison. The study was carried out in a nursery through simulation of the saline and acidic soil environments.

9.2 Methodology

9.2.1 Place of the study

The study was carried out in a green house facility at the Forest and Wood Science Department, University of Stellenbosch from December the 18th of April 2007 to the 3rd of October 2007. The green house was equipped with automated irrigation and cooling systems. It also has 80% shade-netting side walls polycarbonate roof end walls 40% shade-netting ceiling, and a concrete floor.

9.2.2 Fertilisers and soils

The N-Sucrolin lignin, modified at a pilot plant in Freienhufen, Germany was compared to NOVIHUM™ obtained from Novihum GmbH, Großräschen/OT Freienhufen, Germany. The properties of the materials are given in table 9-1 below.

Table 9-1: Elemental properties of the test materials (values given on a dry matter basis).

N-lignin	Nitrogen [%]*				total C	C/N	total S
	total	NH_4^+-N	NH_2-N	Sob-N	[%]	ratio	[%]
N-Sucrolin	4.95	29.49	9.49	61.02	58.70	11.8	0.54
NOVIHUM™	5.34	30.3	12.4	57.30	63.90	12.0	0.74

*The NH_4^+-N, NH_2-N and the Sob-N were calculated as a percentage of the total nitrogen.

The moisture contents of the materials were ascertained using TAPPI standard method T12wd-82 prior to application. The performances of the materials were measured against the ammonium sulphate (21% N, 24% S) obtained from Agricol; a local fertiliser supply company based in Brackenfell. Phosphorus was applied as super phosphate obtainable from Agrimark, Stellenbosch, and potassium as KCl (50% K) obtainable from a local chemical supply company.

An acidic sandy soil was collected form a Mountain to Forest (MTO) plantation in Grabouw. 20cm of topsoil was removed before the sandy soil was collected. An alkaline sandy soil was collected from Big Bay near Blouberg Strand, Cape Town. The compositions of the two soils are given in table 9-2 below.

Table 9-2: Soil properties.

Soil origin	Depth (cm)	pH (KCl)	Resistance. (Ohm)	H^+ (cmol/kg)	Stone (Volume %)	P mg/kg	K mg/kg	Exchangeable cations (cmol/kg) Na	K	Ca	Mg	C %
Big Bay	0	9.0	1700	-	1	4	12	0.10	0.03	11.78	0.31	0.13
MTO	20	3.3	8150	0.60	2	1	5	0.03	0.01	0.16	0.06	0.20

The soil and the fertilising materials were mixed prior to placing inside the growing trays.

9.2.3 Experimental design

The nutrient and growth response of *Eucalyptus marcarthurii* and *Acacia mearnsii* nursery container tree seedlings was tested as summarised in table 9-3 below.

Table 9-3: Experimental design of *Eucalyptus marcarthurii* and *Acacia mearnsii* tree seedlings grown on soil treated with the N-fertilising materials and ammonium sulphate.

Soil Type	Tree species	Fertiliser*	Treatment level	n
Saline soil	*Eucalyptus marcarthurii*	Control	0	14
Saline soil	*Eucalyptus marcarthurii*	$(NH_4)_2SO_4$	-	14
Saline soil	*Eucalyptus marcarthurii*	NOVIHUM™	0.5%	14
Saline soil	*Eucalyptus marcarthurii*	NOVIHUM™	1.0%	14
Saline soil	*Eucalyptus marcarthurii*	NOVIHUM™	1.5%	14
Saline soil	*Eucalyptus marcarthurii*	NOVIHUM™	2.0%	14
Saline soil	*Eucalyptus marcarthurii*	N-Sucrolin	0.5%	14
Saline soil	*Eucalyptus marcarthurii*	N-Sucrolin	1.0%	14
Saline soil	*Eucalyptus marcarthurii*	N-Sucrolin	1.5%	14
Saline soil	*Eucalyptus marcarthurii*	N-Sucrolin	2.0%	14
Saline soil	*Acacia mearnsii*	$(NH_4)_2SO_4$	-	14
Saline soil	*Acacia mearnsii*	NOVIHUM™	0.5%	14
Saline soil	*Acacia mearnsii*	NOVIHUM™	1.0%	14
Saline soil	*Acacia mearnsii*	NOVIHUM™	1.5%	14
Saline soil	*Acacia mearnsii*	NOVIHUM™	2.0%	14

Table 9-3: Experimental design of *Eucalyptus marcarthurii* and *Acacia mearnsii* tree seedlings grown on soil treated with the N-fertilising materials and ammonium sulphate (continued).

Soil Type	Tree species	Fertiliser*	Treatment level	n
Saline soil	*Acacia mearnsii*	N-Sucrolin	0.5%	14
Saline soil	*Acacia mearnsii*	N-Sucrolin	1.0%	14
Saline soil	*Acacia mearnsii*	N-Sucrolin	1.5%	14
Saline soil	*Acacia mearnsii*	N-Sucrolin	2.0%	14
Acidic soil	*Eucalyptus marcarthurii*	Control	0	14
Acidic soil	*Eucalyptus marcarthurii*	$(NH_4)_2SO_4$	-	14
Acidic soil	*Eucalyptus marcarthurii*	NOVIHUM™	0.5%	14
Acidic soil	*Eucalyptus marcarthurii*	NOVIHUM™	1.0%	14
Acidic soil	*Eucalyptus marcarthurii*	NOVIHUM™	1.5%	14
Acidic soil	*Eucalyptus marcarthurii*	NOVIHUM™	2.0%	14
Acidic soil	*Eucalyptus marcarthurii*	N-Sucrolin	0.5%	14
Acidic soil	*Eucalyptus marcarthurii*	N-Sucrolin	1.0%	14
Acidic soil	*Eucalyptus marcarthurii*	N-Sucrolin	1.5%	14
Acidic soil	*Eucalyptus marcarthurii*	N-Sucrolin	2.0%	14
Acidic soil	*Acacia mearnsii*	$(NH_4)_2SO_4$	-	14
Acidic soil	*Acacia mearnsii*	NOVIHUM™	0.5%	14
Acidic soil	*Acacia mearnsii*	NOVIHUM™	1.0%	14
Acidic soil	*Acacia mearnsii*	NOVIHUM™	1.5%	14
Acidic soil	*Acacia mearnsii*	NOVIHUM™	2.0%	14
Acidic soil	*Acacia mearnsii*	N-Sucrolin	0.5%	14
Acidic soil	*Acacia mearnsii*	N-Sucrolin	1.0%	14
Acidic soil	*Acacia mearnsii*	N-Sucrolin	1.5%	14
Acidic soil	*Acacia mearnsii*	N-Sucrolin	2.0%	14
Total number of seedlings				560

(*) $(NH_4)_2SO_4$ was applied at a rate equivalent at 30% of NOVIHUM™.

9.2.4 Fertilising

Phosphorus and potassium were added in each inset as recommended in Handreck and Black (1994) i.e. the ratios of phosphorus to nitrogen (P:N ratio) and potassium to nitrogen (K:N ratio) were 0.14 and 0.52 respectively. Nitrogen in each inset was applied as described in tables 9-4a, b and c below. The bulk densities of the two soils were different (factor = 1.059) and hence the different values of nitrogen. In addition, the nitrogen contents were also different as shown in table 9-1 above. The amount of nitrogen applied per inset was calculated as described in chapter 6. The amount of ammonium sulphate added was equal to 30% of 0.5% variant of NOVIHUM™. This value is called the effective equivalent nitrogen (Katzur et al. 2003a).

Table 9-4 (a): Nitrogen application rates of NOVIHUM™ (n=14).

Soil	NOVIHUM™			
	0.5 Mass %	1.0 Mass %	1.5 Mass %	2% Mass
Variants)	**V3**	**V4**	**V5**	**V6**
Acidic soil	48.06 mgN/inset	96.31 mgN/inset	144.20 mgN/inset	192.27 mgN/inset
Saline soil	45.39 mgN/inset	90.78 mgN/inset	136.17 mgN/inset	181.56 mgN/inset

Table 9-4 (b): Nitrogen application rates of N-Sucrolin (n=14).

Soil	N-Sucrolin			
	0.5 Mass %	1.0 Mass %	1.5 Mass %	2% Mass
Variants)	**V3**	**V4**	**V5**	**V6**
Acidic soil	30.40 mgN/inset	60.80 mgN/inset	91.2 mgN/inset	121.62 mgN/inset
Saline soil	28.71 mgN/inset	57.42 mgN/inset	86.17 mgN/inset	114.84 mgN/inset

Table 9-4 (c): Nitrogen application rates of $(NH_4)_2SO_4$ as well as the control (n=14).

Soil	Control	$(NH_4)_2SO_4$
Variants	**V1**	**V2**
Acidic soil	0g mgN/inset	14.42 mgN/inset
Saline soil	0g mgN/inset t	13.62 mgN/inset

9.2.5 Seed

Tree seed i.e. *Eucalyptus marcarthurii* and *Acacia mearnsii* were kindly donated by the Institute for Commercial Forestry Research (ICFR), Scottsville, South Africa. The seeds were planted into seedling trays and were allowed to germinate for seven weeks. After the germination period, the seedlings were transplanted into 10 cm diameter by 10 cm height polyvinyl pots with four perforation holes at the bottom. Each pot could hold 2 kg and 2.1 kg the saline soil and of the acidic soil respectively. The nutrient application in the pots was exactly similar to that of the trays.

9.2.6 Maintenance

The pots were given water for 2 minutes twice a day using an automatic watering system.

9.2.7 Assessment

The seedlings were assessed 4 times during the experimental period i.e. after 16, 20, 24 and at the end of the experiment (after 28 weeks). At end of the experiment roots of seedlings were washed. Containers were immersed in water-logged buckets, and the containers were slipped off without disturbing the roots. The roots of each seedling were washed separately in a bucket. Water used for washing was put through a 1.7mm sieve to recover roots broken off during washing as described by Motoma (1998).

9.2.8 Variables measured

To assess seedling quality throughout the experimental period, the following parameters were evaluated:

- Root collar diameter was accurately measured on cotyledon scars with an electronic calliper.
- The dry mass of the root and shoot systems of each seedling was determined after drying them at 70°C until constant weight.
- A macro-nutrient analysis of the roots and shoots was carried out.

9.2.9 Data presentation and statistical analysis

Data were entered into a spreadsheet and the means were computed. The effects of treatment, treatment level, tree species, time and their interaction was evaluated in a Statistica 7 computer program. After providing significant differences at $p \leq 0.05$, a four way Analysis of Variation (ANOVA) was used to test significant differences between treatments.

9.3 Results and discussion

9.3.1 General observations

The two soils were distinctly different from each other in terms of pH and as such, there were differences in nutrient mobilisation and availability to the seedlings. Growth of the seedlings in the alkaline soil was not successful as the fertilising materials could not mitigate the effects of the high soil pH (9.0). Figure 9-1 below gives a visual illustration of the tree seedling growth response in the two soils. Due the poor growth

in alkaline soil, this discussion focuses on the performance of N-fertilising materials in the acidic soil.

Figure 9-1: Seedlings grown in alkaline (left) and acidic soil (right).

The two tree species were distinctly different form each other in terms of growth patterns. Their shoots and roots differ morphologically, thus, resulting in different responses to treatment with the fertilising materials. *Acacia mearnsii* had scanty roots, short and extensively branched stems and in general grew slower while *Eucalyptus marcarthurii* grew faster and taller. As such, each tree seedlings' response to treatment with the fertilising materials is discussed separately.

9.3.2 Root collar diameter (RCD) in acidic soil

Eucalyptus marcarthurii

Mean RCD of *Eucalyptus marcarthurii* was not significantly influenced by the type of N-lignin fertilising material treatment (p=0.14805) (figure 9-2). It increased with the age of the seedlings, however, contrary to expectations, the differences observed were not significant (p= 0.1283) (figure 9-3).

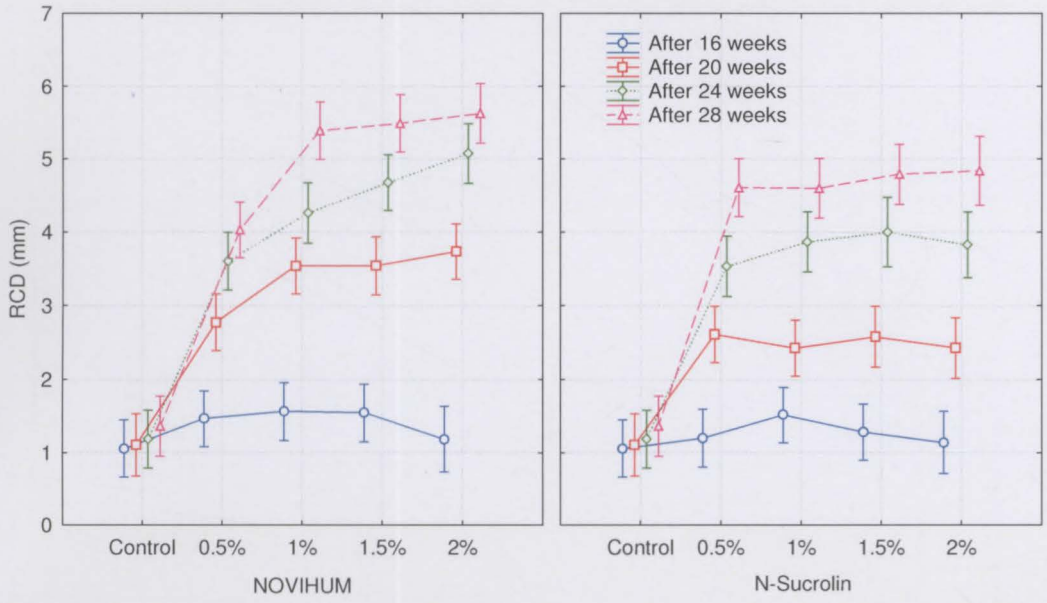

Figure 9-2: Least square mean RCDs of *Eucalyptus marcarthurii* at different N-lignin treatment levels.

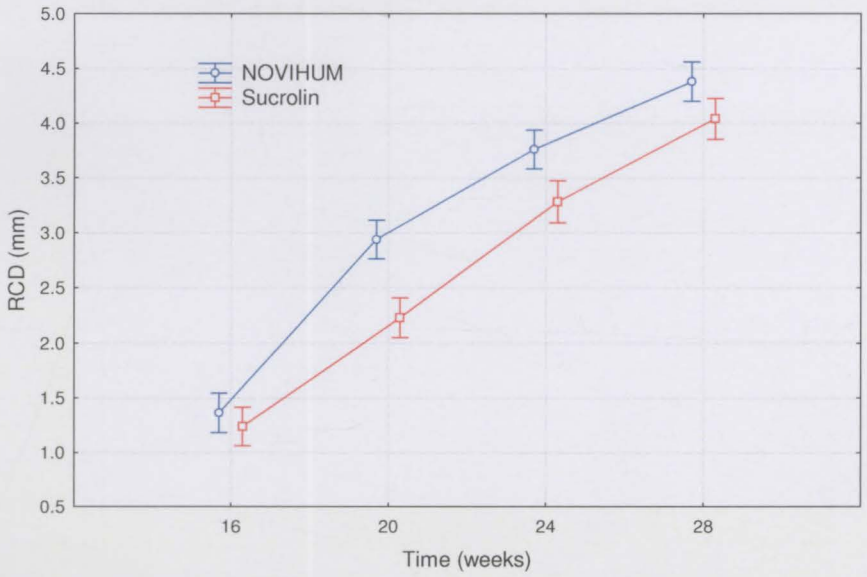

Figure 9-3: Least square mean RCDs of *Eucalyptus marcarthurii* at different time intervals.

In comparison with inorganic ammonium sulphate fertiliser, both the NOVIHUM™ and the N-Sucrolin yielded significantly higher mean RCDs over time (figure 9-4 and 9-5). As discussed in chapter 8, at equivalent nitrogen application, inorganic fertilisers leach some of the nitrogen due to their solubility in water. These observations were also

made by Katzur et al. (2003), further highlighting the advantages of using N-lignins as fertilising materials.

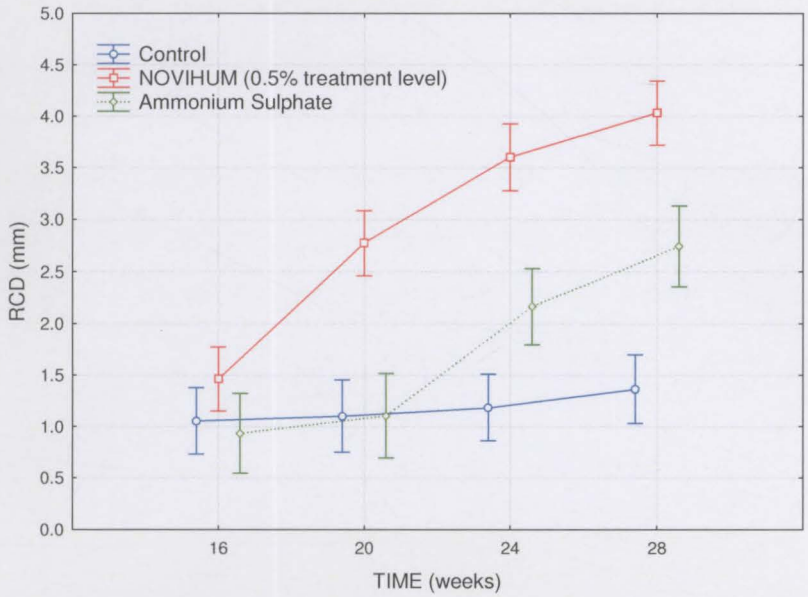

Figure 9-4: Least square mean RCDs of *Eucalyptus marcarthurii* treated with NOVIHUM™ and ammonium sulphate over time.

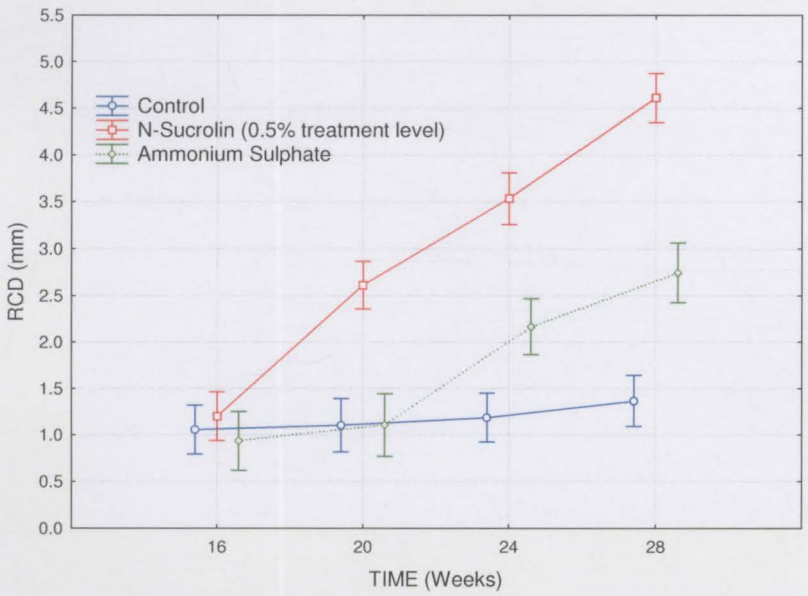

Figure 9-5: Least square mean RCDs of *Eucalyptus marcarthurii* treated with N-Sucrolin and ammonium sulphate over time.

Acacia mearnsii

Mean RCD of *Acacia mearnsii* seemed to decrease with N-lignin treatment level. As *Acacia mearnsii* fixes its own nitrogen, the excess nitrogen i.e. as the treatment levels are increased, could be toxic to the roots and hence resulting in low yield. As seen figure 9-6, this was more so with N-Sucrolin than it was for NOVIHUM™ and the differences in mean RCD were significant (p=0.000). Contrary to observations with eucalypts, it significantly increased with time (p=0.3385) as expected (figure 9-7).

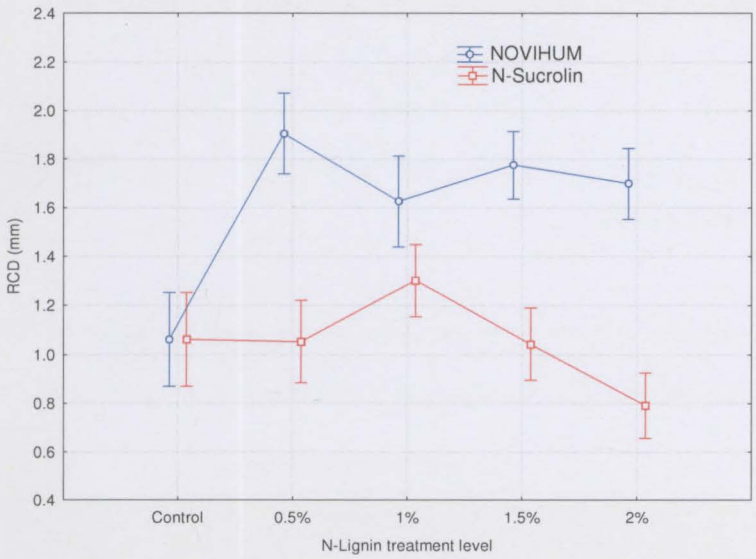

Figure 9-6: Least square mean RCDs of *Acacia mearnsii* at different N-lignin treatment levels.

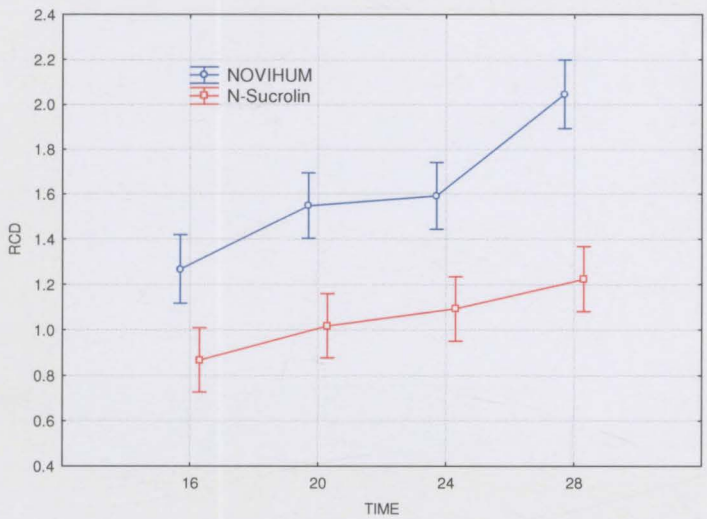

Figure 9-7: Least square mean RCDs of *Acacia mearnsii* at different time intervals.

9.3.3 Foliage dry matter (DM) yield in acidic soil

As discussed above the, the two tree species are morphologically distinctly different, and hence different growth patterns were observed leading to different responses to treatments. Figure 9-8 below further illustrates this phenomenon.

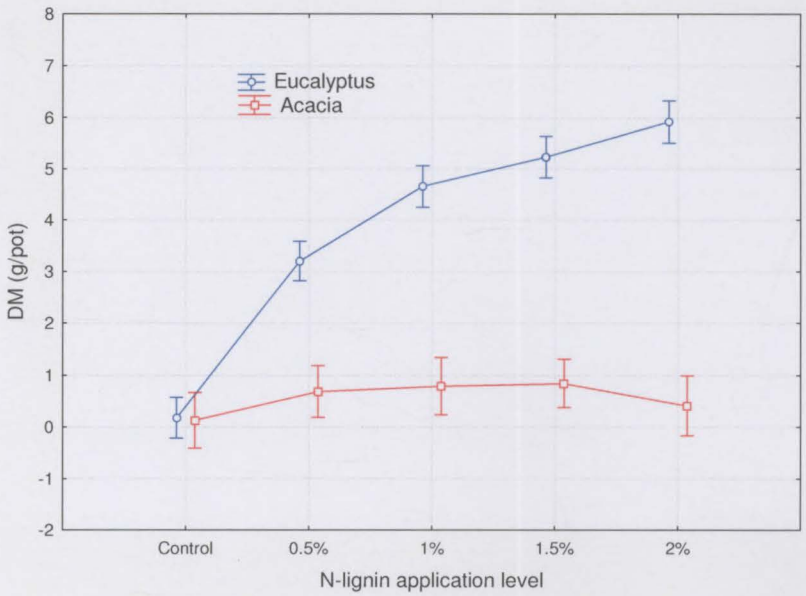

Figure 9-8: Mean foliage DM yield of *Eucalyptus marcarthurii* and *Acacia mearnsii* treated with N-Sucrolin at the end of the experiment (after 28 weeks).

Eucalyptus marcarthurii

Foliage DM yield of *Eucalyptus marcarthurii* increased with N-lignin treatment level, however, the yields were not significantly different (p= 0.10377) between the N-Sucrolin and the NOVIHUM™ (figure 9-9). The N contents of the two materials were very close and hence this kind of result was expected. On the contrary, as illustrated in figures 9-10 and 9-11, the DM yield with ammonium sulphate was significantly lower than both NOVIHUM™ and N-Sucrolin treatment at equivalent N application. This was also described by Katzur et al. (2003).

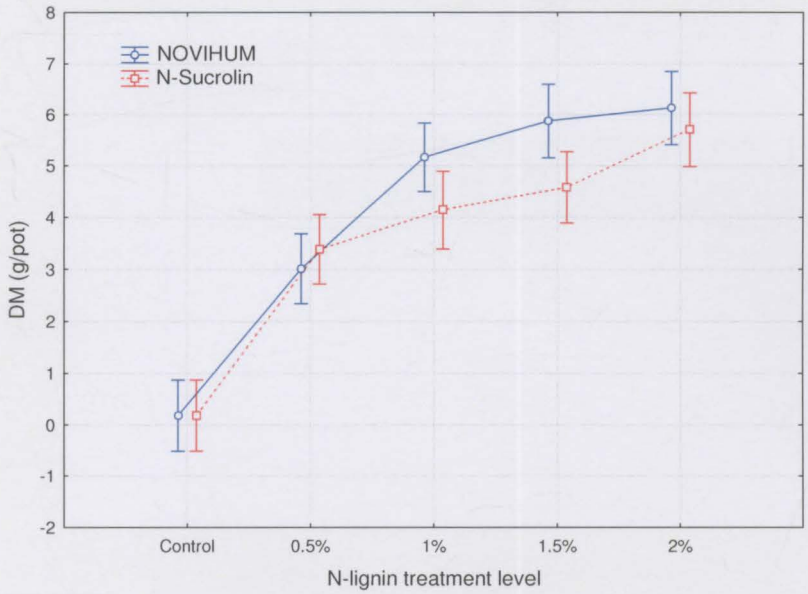

Figure 9-9: Least square mean foliage DM yields of *Eucalyptus marcarthurii* at different treatment levels of NOVIHUM™ and N-Sucrolin.

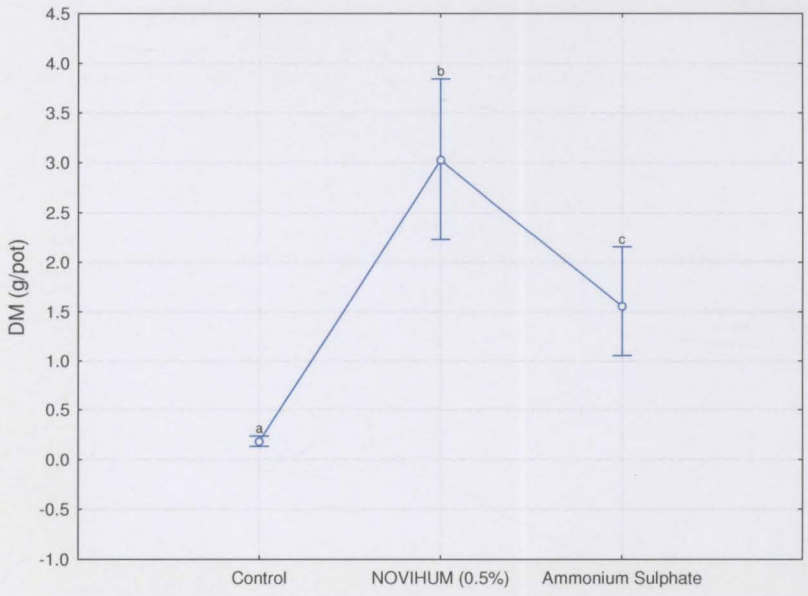

Figure 9-10: Bootstrap mean foliage DM yields of *Eucalyptus marcarthurii* treated with NOVIHUM™ and ammonium sulphate.

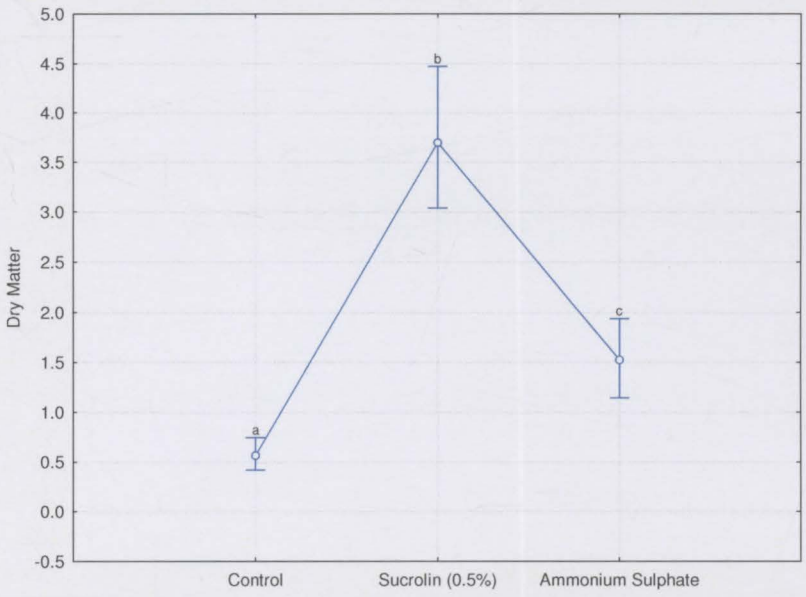

Figure 9-11: Bootstrap mean foliage DM yields of *Eucalyptus marcarthurii* treated with N-Sucrolin and ammonium sulphate.

Acacia mearnsii

As observed with the RCD, the foliage DM yields of the NOVIHUM and N-Sucrolin were reduced at the as N-treatment level increased (figure 9-12). The N-Sucrolin yielded significantly lower results (p=0.01133) compared to NOVIHUM™.

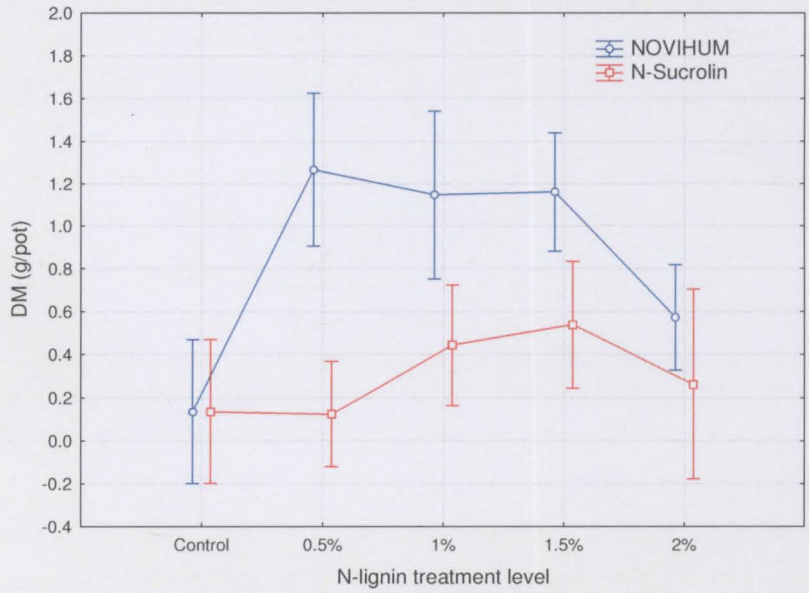

Figure 9-12: Least square mean foliage DM yields of *Acacia mearnsii* at different treatment levels of NOVIHUM™ and N-Sucrolin.

9.3.4 Root dry matter (DM) yield in acidic soil

The root dry matter yield of the Eucalyptus was distictyly different from that of the acacia as figure 9.13 below demonstrates. This was due to the fact that the two tree species were morphologicaly different and as such had different bulk densities as already discussed above.

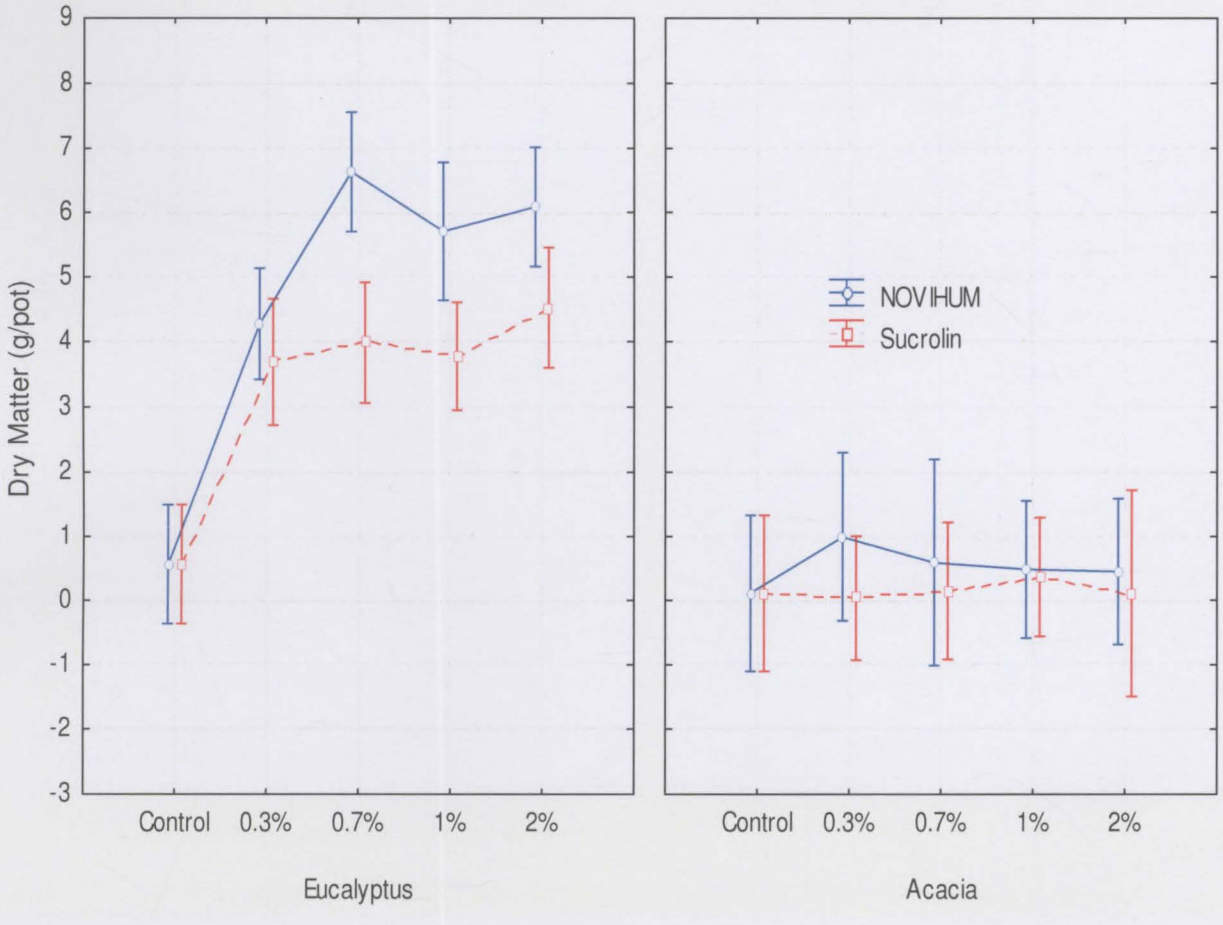

Figure 9-13: Least square mean DM yields of *Eucalyptus marcarthurii* at different N-lignin treatment levels.

The DM yield of Eucalyptus grown on soil fertilised with Sucrolin was not significantly different (p=0.00049) from that grown on NOVIHUM™ (see figure 9-14). Although this was expected, it was contrary to observations in the foliage DM yields. If the N-contents of the two materials were similar, the yields were also expected to be similar.

However, there could be also material properties related to the type and source of material which could influence the results. However, this study only focused on N contents and C/N ratios and as such the comparison was limited to these two parameters.

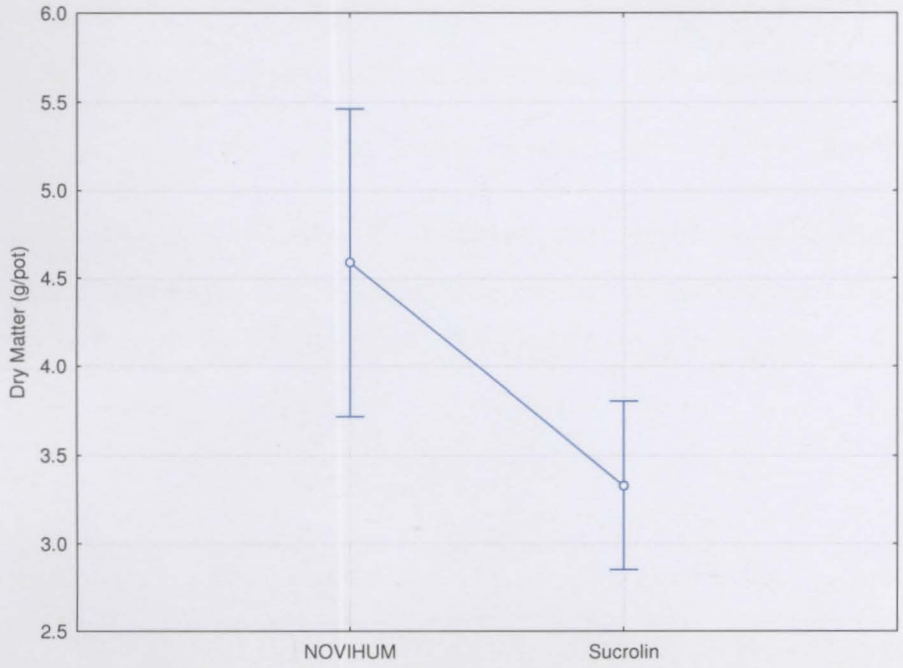

Figure 9-14: Least square mean DM yield of *Ecualyptus marcarthuri*i grown on soil trated with N-lignins.

9.3.5 Nutrient accumulation in seedlings

Tables 9-5 below compare the macro and micro-nutrient contents of foliage and roots of the two tree species under different N-lignin treatment levels. Previous studies have shown that the higher the nitrogen applied in the soil, the higher the accumulation in crops and the higher the amount susceptible to leaching. However, the studies have also shown that nitrogen use efficiency decreased with increasing nitrogen application (Katzur et al. 2003), (see also chapter 8). The two tree species were distinctly different in terms of morphology and growth patterns and yielded different dry mass and root collar diameters. The results indicate that *Acacia mearnsii* generally accumulated more nutrients in the leaves and the roots than *Eucalyptus marcarthurii.* The expected trend

of increasing nitrogen (in the plant) with increasing application level is more pronounced in the NOVIHUM™ treatment of *Acacia mearnsii* (correlation coefficient between nitrogen content and nitrogen application = 0.941317) (foliage). The trend was not as high in the NOVIHUM™ treatment (foliage) (correlation coefficient =0.767205) in *Eucalyptus marcarthurii.* A balance in nutrient allocation between the foliage and the roots was noticeable in *Eucalyptus marcarthurii.* Contrary to this, a distinct partitioning of nutrient allocation between foliage and roots was observed in *Acacia mearnsii.*

At equivalent nitrogen application, the treatment with ammonium sulphate showed more nitrogen in the roots than in the leaves. No distinct differences were observed between treatment with NOVIHUM™ and N-Sucrolin in both tree species.

Table 9-5: Macro content (N, P and K) of foliage and roots in *Eucalyptus marcarthurii* and *Acacia mearnsii* seedlings (values given on a dry matter basis).

Eucalyptus marcarthurii						*Acacia mearnsii**					
Treatment	Foliage or Roots	Treatment Level	N (%)	P (%)	K (%)	Treatment	Foliage or Roots	Treatment Level	N	P	K
NOVIHUM™	Foliage	2.0%	2.57	0.48	1.19	NOVIHUM™	Foliage	2.0%	4.35	1.13	2.41
	Foliage	1.5%	1.45	0.40	0.92		Foliage	1.5%	4.01	0.96	2.09
	Foliage	1.0%	1.95	0.34	1.16		Foliage	1.0%	3.94	0.70	1.92
	Foliage	0.5%	1.65	0.36	1.31		Foliage	0.5%	3.23	0.73	1.72
N-Sucrolin	Foliage	2.0%	2.96	0.45	1.21	N-Sucrolin	Foliage	2.0%	3.23	0.53	1.20
	Foliage	1.5%	1.57	0.35	1.43		Foliage	1.5%	4.05	1.02	2.51
	Foliage	1.0%	2.27	0.39	1.40		Foliage	1.0%	4.70	1.02	2.48
NOVIHUM™	Roots	2.0%	1.25	0.35	0.45	NOVIHUM™	Roots	2.0%	2.33	1.36	1.77
	Roots	1.5%	1.25	0.39	0.60		Roots	1.5%	2.61	1.84	2.07
	Roots	1.0%	1.17	0.35	0.58		Roots	1.0%	2.28	1.36	2.04
	Roots	0.5%	0.79	0.26	0.80		Roots	0.5%	2.08	1.09	1.78
N-Sucrolin	Roots	2.0%	1.99	0.43	0.62	N-Sucrolin	Roots	2.0%	4.75	0.52	1.26
	Roots	1.5%	2.67	0.46	1.41		Roots	0.5%	1.30	0.43	0.72
	Roots	1.0%	1.01	0.28	0.59				-	-	-
	Roots	0.5%	0.85	0.32	0.71				-	-	-
									-	-	-
AMM SULPH	Foliage	AMM SULPH	1.22	0.25	0.65				-	-	-
	Roots	AMM SULPH	1.66	0.32	1.78				-	-	-
Control	Foliage	0.0%	1.17	0.82	2.01				-		
	Roots	0.0%	0.51	0.32	0.56				-	-	-

* - control and ammonium sulphate not measured.

9.4 Conclusion

None of the fertilising materials could mitigate the effects of the high pH (9.0) in the alkaline soil and resulted in that the growth of the tree seedlings was not successful. Both the N-Sucrolin and the NOVIHUM™ improved the growth of tree seedlings. In the acidic soil, the growth pattern of the *Acacia mearnsii,* a nitrogen fixer was distinctly different from *Eucalyptus marcarthurii*, a non-nitrogen fixer. The response of *Eucalyptus marcarthurii* in soil treated with N-Sucrolin was slightly lower in terms of root collar diameter and dry mass yield than that of NOVIHUM™ treatment; however, the differences were not significant. At equivalent nitrogen, the N-Sucrolin and the NOVIHUM™ had relatively higher dry mass yields and higher root collar diameters than ammonium sulphate due to the susceptibility of ammonium sulphate to leaching. Contrary to this, the N-lignins slowly released their nitrogen in the soil. The response of *Acacia mearnsii* in soil treated with N-lignins decreased. The N-Sucrolin yielded significantly lower root collar diameter and dry matter compared to the NOVIHUM™. Nutrient accumulation in the plant was much higher in *Acacia mearnsii* than in *Eucalyptus marcarthurii* mostly as a result of this tree species being able to fix its own nitrogen. It also showed a higher accumulation of nutrients in the foliage than in the roots while in *Eucalyptus marcarthurii*, the portioning was not so distinct. Yields with both N-Sucrolin and NOVIHUM™ increased with N application level. However, a value between the 0.5% to 1% application rates would lead to better nitrogen use efficiency as also reported in literature.

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10.0 Conclusion and perspectives

10.1 Conclusion

The results from the current study provide a basis for the development of organic based, slow nitrogen release, soil improving materials from technical lignins i.e. Sucrolin, bagasse lignocellulosic residue and calcium lignosulphonate obtained from South African sources using the patented procedure NOVIHUM™ manufacturing procedure. In addition, the study indicates possible areas of application of the N-lignins. The research provided an understanding of the following aspects:

- physical and chemical properties of the technical lignins
- the total nitrogen contents and C/N ratios that can be achieved and how they can be improved
- the proportions of N-binding forms i.e. as immediately plant available, mid-term and long term plant available
- the effect of ammoxidation on the functional groups of technical lignins
- the nitrogen containing lignins compounds as elucidated by a combination of analytical techniques and how the techniques varied from each other in terms of sensitivity
- the ability of the N-lignin products in producing biomass (crops) in quantities that are similar to the standard reference product NOVIHUM™ as well as how they compare with inorganic fertilisers such as urea and ammonium sulphate
- the appropriate amounts by which the N-lignins should be applied in order to improve nitrogen use efficiency in crops and avoid excessive nitrogen leaching into the soil.

The study demonstrated the feasibility of utilizing the selected raw materials. There were differences in response towards nitrogen incorporation which were related to elemental, functional group content as well as well as technical lignin type. The study

revealed that the Sucrolin was a technical lignin of relatively high purity as it had distinctly lower quantities of contaminants such as ash, sugars and sulphur compared to the other selected technical lignins. It was also found to be highly oxidised as shown by its higher content of carboxyl groups. This augured well for the oxidative ammonolysis process as oxidation is central to the success of nitrogen incorporation into technical lignins. It also had comparatively high carbon content and Pyrolysis GCMS showed that it had a higher content of phenolic moieties (and low carbohydrate contents) whose unsaturated *pi* electronic structure is essential in the modification of ligneous raw materials into nitrogen-enriched humus-like products. On the contrary, the selected calcium lignosulphonate was shown to have relatively high amount of ash and reducing sugar content which were typical of the sulphite pulping process. The lignocellulosic residue also had a low lignin content to start with due to its relatively high cellulose content. Its ash content was also a higher compared to Sucrolin.

Upon modification at a laboratory scale with the patented procedure, the Sucrolin showed the highest amount of incorporated nitrogen than the other two selected technical lignins as well as Kraft lignin, Indulin AT, selected for comparison. Its nitrogen content was also higher than that of NOVIHUM™, the standard reference product manufactured at pilot scale. It showed satisfactory distribution of the various proportions of nitrogen binding forms comparable to other N-lignins as reported in literature. The N-content obtained with the lignocellulosic residue was lower due to its relatively high cellulose content but could be increased by pre-oxidising the material with hydrogen peroxide before ammoxidation. Its distribution of the various proportions of nitrogen binding forms was also acceptable. The N-calcium lignosulphonate showed a lower total N-content than Sucrolin but in the acceptable range as other N-lignins reported in literature. However, it had comparatively high NH_4^+ and low *sob*-N contents, due to its high sulphur content.

Under ambient pressure conditions, effective oxygen feeding to the reaction mixture instead of air resulted in increased nitrogen contents in all three candidate N-lignins. The ammoxidation process itself is a mild oxidation and re-arrangement of the

functional groups, such as carboxylic groups. ^{31}P NMR showed that after modification, the content of primary alcohol groups decreased indicating that some condensation reactions or the cleavage of lignin side chains had taken place.

The combination of analytical techniques used, provided more insight into the nature of N-binding forms of N containing compounds in N-lignins. While it was difficult to assign peaks to N containing heterocyclic compounds with pyrolysis GCMS due to low sensitivity caused by small and broad signals, more signals could be detected with ^{15}N CPMAS. In agreement with results obtained with wet chemical methods, the N-Sucrolin showed a similar distribution of the N-binding forms as the NOVIHUM™ as also shown by XPS.

The N-Sucrolin showed similar biomass yields as NOVIHUM™, the standard reference product, on plant performance evaluation tests with yellow mustard (*Sinapis alba)* in a green house extended over two growing periods. Given the fact that the N-Sucrolin sample tested had a lower nitrogen content than NOVIHUM™, it can be considered very effective from the nutrient efficiency and economic perspective. No phytotoxic effects were observed with this material. Dry mass yields from N-bagasse lignocellulosic residue soil treatments were similar to N-Sucrolin and NOVIHUM™ in the first growth period despite its relatively low nitrogen content. The lower nitrogen content resulted in a comparatively lower yield in the second growth period. However, the nitrogen content could be increased by pre-activation of the material with hydrogen peroxide. Supplementary addition of nitrogen to the N-bagasse lignocellulosic residue in the soil did not improve the yield but rather showed faster growth and taller stems. Phytotoxic effects as a result of soil treatment with this material were not observed. Soil treatments with N-modified calcium lignosulphonate resulted in very low yields even with supplementary addition of urea. Physiological studies showed that the low yield was not related to the nitrogen content as phytohormonal analysis showed a similar behaviour for the three N-lignins as well as NOVIHUM™, the standard reference product.

In further tree seedling tests on acidic (typical of post-mining areas) and saline soil (typical of sand dunes of the west coast of South Africa), N-Sucrolin showed similar yields as NOVIHUM™, and higher yields than ammonium sulphate, as a result of its longer lasting effect on the soil. However, in alkaline soil, neither the N-Sucrolin nor the NOVIHUM™ could mitigate the effects of high pH, and successful growth of plant was not possible.

The results of the study also confirmed that nitrogen was most efficiently used in the lower application range i.e. 0.3-1% (mass% N-lignin/mass % soil). At higher rates, lower biomass yields were observed due to the low survival rate resulting from the toxic effect of excess nitrogen. At equivalent nitrogen, inorganic fertilisers were shown to loose high amounts of nitrogen in percolation water, confirming the advantage of the slow nitrogen release ability of N-lignins.

10.2 Perspectives

It has been quoted in many publications that South Africa loses approximately 300 to 400 million tons of soil to soil erosion on an annual basis. Soil degradation through soil erosion and other forms have been estimated to cost South Africa nearly R2 billion per annum. South Africa loses an estimated 30000 tons of nitrogen, 26400 tons of phosphorus and 363000 tons of potassium to soil erosion on an annual basis. Arresting the process of soil degradation requires tremendous and concerted from various stake holders i.e. the state, communities, agricultural industries, educational and research institutions.

Central to the problem of soil degradation is the loss of soil organic matter which is vital for numerous soil functions as well as its structure. The use of agricultural residues such as composts and sewage sludge does not adequately solve the problem of the lack of soil organic matter. This is mainly due to the fact that their composition varies widely and they could be sources of pollutants. The use of N-lignins could in an eco-friendly approach be considered as an alternative way of addressing the soil organic matter needs of degraded environments. In addition, they also contain nitrogen that

could be slowly released into the soil and thereby prevent excessive leaching of nitrogen to ground water. The slow nitrogen release ability of N-lignins could also reduce application cost especially considering that crops can be replanted in the same soil without additional nitrogen inputs. The use of N-lignins could be further considered as a way of returning industrial by-products (technical lignin) in an eco-friendly approach to the soil as a fertilising material.

The results of the study clearly demonstrated the success of Sucrolin as a raw material for preparing N-lignins. Its performance on acid soil could pave a way for its use in the field of soil rehabilitation, one of the intended areas of use for N-lignins. The results obtained with the bagasse lignocellulosic residue are quite encouraging. They open up possible applications for this material produced in large quantities around the world. However, the ammoxidation of calcium lignosulphonate was not successful due to its chemical composition. Further research on the use of calcium lignosulphonate should focus on the suspected phytotoxic compounds which have been described by other workers as five membered, heterocyclic nitrogen containing compounds. Plant tests have shown that stunted growth and low yield, resulting from the use of this material were not related to the nitrogen inadequacy as the product prepared from this material had similar nitrogen content and C/N ratio as N-lignins prepared reported in literature. Physiological studies have also showed similar behaviour of this material as other N-lignins.